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(54) Title: GALANIN RECEPTOR GalR2

(57) Abstract

The present invention provides a novel galanin receptor protein, the GalR2 receptor. Also provided are the nucleic acid sequences encoding this novel receptor protein as well as methods for using this protein and its nucleic acid sequence, and methods useful for developing and identifying compounds for the treatment of diseases and disorders in which galanin is implicated. The importance of this discovery is manifested in the effects of galanin, which include antinociceptive activity, smooth muscle contraction, cardiovascular activity, pituitary hormone release, cognition, and increased food intake. Thus, this receptor protein is useful for screening for galanin agonist and antagonist activity for controlling these conditions.

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GALANIN RECEPTOR GalR2

This application claims priority from U.S. Provisional Application No. 60/019946, filed June 5, 1996.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a novel neuropeptide galanin receptor and its nucleic acid sequence.

2. Description of the Related Art

Galanin is a 29 amino acid peptide hormone (30 amino acids in human) which is present in a wide range of central and peripheral tissues. Skofitsch, *Peptides* 6, 509 (1985); Merchenthaler, *Prog. Neurobiol.* 40, 711 (1993). Galanin is involved in many diverse physiological functions. Galanin is known to regulate the secretion of both endocrine and exocrine hormones. Galanin inhibits insulin secretion from pancreatic beta cells, and can inhibit pancreatic amylase secretion; in the stomach, galanin inhibits gastrin and somatostatin secretion. Galanin stimulates VIP (vasoactive intestinal protein) release from the hypothalamus, prolactin and growth hormone release from the pituitary; and inhibits the secretion of ACTH in the hypothalamus. Furthermore, the secretion of neurotransmitters can be modulated. For example, galanin can inhibit the release of histamine and norepinephrine in the hypothalamus. Other secondary messenger systems are also regulated: galanin can either stimulate or inhibit intracellular cAMP accumulation; is involved in the closure of N- and L-type voltage-sensitive calcium channels, and in the opening of ATP-sensitive and -insensitive potassium channels; and has been shown to stimulate the release of calcium from intracellular stores. Moreover, galanin is involved in the inhibition of acetylcholine release and the inhibition of muscarinic receptor-mediated phosphoinositide turnover. Bartfai, *Crit. Rev. Neurobiol.* 7, 229 (1993)

Galanin is implicated in the modulation of many cognitive and sensory functions. Galanin has potent antinociceptive effects, and can impair performance in one-trial learning, t-maze, and swim maze learning and memory paradigms. Its inhibition of the anoxic release of glutamate, as well as its inhibitory actions on cholinergic function suggest a role in neuroprotection, and in the development of Alzheimer's Disease. Crawley, *Regulatory Peptides* 59, 1 (1995). Galanin is known to induce feeding in rodents and, in contrast with

the effects of Neuropeptide Y on feeding, galanin increases preference for fat intake. Akabayashi, *Proc. Natl. Acad. Sci. USA* **91,** 10375 (1994). Galanin is also involved in the regulation of gastrointestinal smooth muscle contraction. Because of the important role of galanin in these many physiological processes, there is a strong need to further develop materials and methods for investigating the mechanistic behavior of the receptors and for treating diseased and other abnormal states associated with these physiological processes.

Pharmacological data suggest the existence of several galanin receptor subtypes. Wynick, *Proc. Natl. Acad. Sci. USA* **90,** 4231 (1990); Zen-Fa, *J. Pharmacol. Exp. Ther.* **272,** 371 (1995). Galanin receptors are known to be linked to the G_i proteins, and there is some evidence that certain galanin receptor subtypes may be linked to cholera toxin-sensitive G_s proteins. Gillison, *Diabetes* **43,** 24 (1994); Chen, *Am. J. Physiol.* **266,** G113 (1994). One galanin receptor has been cloned and it is a member of the seven transmembrane (7TMD) class of G protein-linked receptors. It has been designated as GalR1. Habert-Ortoli, *Proc. Natl. Acad. Sci. USA* **91,** 9780; WO95/22608. In addition to this human GalR1 receptor, the GalR1 receptor has been obtained from rat. Burgevin, *J. Mol. Neurosci.* **6,** 33 (1995). The *in vivo* functions mediated through this cloned GalR1 receptor have not yet been elucidated. EP-0711830-A2 disclose a closely-related GalR1 sequence, differing in that Cys15→Trp is varied.

SUMMARY OF THE INVENTION

The present invention provides a novel galanin receptor protein, the GalR2 receptor. Also provided are the nucleic acid sequences encoding this novel receptor protein as well as methods for using this protein and its nucleic acid sequence, and methods useful for developing and identifying compounds for the treatment of diseases and disorders in which galanin is implicated. The importance of this discovery is manifested in the effects of galanin, which include antinociceptive activity, smooth muscle contraction, cardiovascular activity, pituitary hormone release, cognition, and increased food intake. Thus, this receptor protein is useful for screening for galanin agonist and antagonist activity for controlling these conditions.

In one aspect of the present invention, we provide isolated nucleic acid sequences for a novel galanin receptor, the GalR2 receptor. In particular, we provide the cDNA sequences encoding the complete rat receptor and partial sequences of the human receptor. These nucleic acid sequences have a variety of uses. For example, they are useful for making

vectors and for transforming cells, both of which are ultimately useful for production of the GalR2 receptor protein. They are also useful as scientific research tools for developing nucleic acid probes for determining receptor expression levels, *e.g.*, to identify diseased or otherwise abnormal states. They are useful for developing analytical tools such as antisense oligonucleotides for selectively inhibiting expression of the receptor gene to determine physiological responses. The present invention can also be used to isolate the homologous nucleic acid sequence of other species, such as human, primate, dog, mouse, etc.

In another aspect of the present invention, we provide a homogeneous composition comprising the receptor GalR2 protein. The protein is useful for screening drugs for agonist and antagonist activity, and, therefore, for screening for drugs useful in regulating physiological responses associated with the GalR2 receptor. Specifically, antagonists to the GalR2 receptor could be used to treat obesity and diabetes by reducing appetite and food consumption, whereas agonists could be used for the treatment of anorexic conditions. Furthermore, drugs could be used to treat Alzheimer's disease, stroke, neuropathic pain, and/or endocrine disorders. The proteins are also useful for developing antibodies for detection of the protein.

Flowing from the foregoing are a number of other aspects of the invention, including (a) vectors, such as plasmids, comprising the receptor GalR2 nucleic acid sequence that may further comprise additional regulatory elements, *e.g.*, promotors, (b) transformed cells that express the GalR2 receptor, (c) nucleic acid probes, (d) antisense oligonucleotides, (e) agonists, (f) antagonists, and (g) transgenic mammals. Further aspects of the invention comprise methods for making and using the foregoing compounds and compositions.

The invention includes polynucleotide molecules coding for a rat or human GalR2, or a galanin binding fragment thereof. A polynucleotide molecule comprising SEQ ID NO:1 or a degenerate variant thereof. A polynucleotide molecule comprising the full-length cDNA, or a degenerate variant thereof, corresponding to the partial sequence shown in SEQ ID NO:5. A purified and isolated rat or human GalR2 protein. The GalR2 protein comprising SEQ ID NO:2. The full-length GalR2 protein, the partial sequence of which is indicated in SEQ ID NO:6. A purified and isolated rat or human GalR2 protein or fragment thereof having galanin binding activity. A polynucleotide molecule coding for a variant of rat GalR2 comprising SEQ ID NO:3, or a galanin binding fragment thereof. A polynucleotide molecule comprising SEQ ID NO:3 or a degenerate variant thereof. A purified and isolated variant of a rat or human GalR2 protein or fragment thereof having galanin binding activity. A purified and

isolated variant of rat GalR2 protein comprising SEQ ID NO:4.

The foregoing merely summarize certain aspects of the present invention and is not intended, nor should it be construed, to limit the invention in any manner. All patents and other publications recited herein are hereby incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is the polynucleotide sequence of rat GalR2 of the invention.

Figure 2 is the amino acid sequence of rat GalR2.

Figures 3-4 are the polynucleotide sequence of the Y107 variant of rat GalR2.

Figure 5 is the amino acid sequence of Y107(omitting putative intron).

Figure 6 is the partial polynucleotide sequence of human GalR2.

Figure 7 is the partial amino acid sequence of human GalR2.

Figure 8 is the representative saturation isotherm of [^{125}I]hGalanin binding to rat GalR2 (clone BMB77) transiently expressed in COS-7 cells. The inset shows the corresponding linear Rosenthal plot. B/F axis on the Rosenthal plot indicates the ratio of Bound to Free radioligand.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention comprises, in part, a novel galanin receptor protein, the GalR2 receptor. Particularly preferred embodiments of the GalR2 receptor are those having an amino acid sequence substantially the same as SEQ ID NO: 2, 4 or 6. As used herein, reference to the GalR2 receptor is meant as a reference to any protein having an amino acid sequence substantially the same as SEQ ID NO: 2, 4 or 6. The present invention also comprises the nucleic acid sequence encoding the GalR2 protein, which nucleic acid sequence is substantially the same as SEQ ID NO: 1, 3 or 5. Receptors SEQ ID NO: 1 and SEQ ID NO: 3 are nucleic acid sequences of rat GalR2 receptors but SEQ ID NO: 3 appears to contain an intronic region; therefore, SEQ ID NO: 1 is the preferred embodiment of the rat GalR2 receptor of this invention. Receptor SEQ ID NO: 5 is the partial nucleic acid sequence of human GalR2. Receptors SEQ ID NO: 2 and 4 are rat GalR2 receptors and appear to be allelic variants. Receptor SEQ ID NO: 6 is the partial amino acid sequence of human GalR2.

As used herein, a protein "having an amino acid sequence substantially the same as SEQ ID NO: x" (where "x" is the number of one of the protein sequences recited herein) means a protein whose amino acid sequence is the same as SEQ ID NO: x or differs only in a

way such that IC_{50}[galanin] as determined according to the method detailed in Example 2, *infra*, are less than or equal to 1 nM. Those skilled in the art will appreciate that conservative substitutions of amino acids can be made without significantly diminishing the protein's affinity for galanin and fragments and analogs thereof. Other substitutions may be made that increase the protein's affinity for these compounds. Making and identifying such proteins is a routine matter given the teachings herein, and can be accomplished, for example, by altering the nucleic acid sequence encoding the protein (as disclosed herein), inserting it into a vector, transforming a cell, expressing the nucleic acid sequence, and measuring the binding affinity of the resulting protein, all as taught herein.

As used herein the term "a molecule having a nucleotide sequence substantially the same as SEQ ID NO: y" (wherein "y" is the number of one of the protein-encoding nucleotide sequences listed in the Sequence Listing) means a nucleic acid encoding a protein "having an amino acid sequence substantially the same as SEQ ID NO: y*" (wherein "y*" is the number of the amino acid sequence for which nucleotide sequence "y" codes) as defined above. This definition is intended to encompass natural allelic variations in the GalR2 sequence. Cloned nucleic acid provided by the present invention may encode GalR2 protein of any species of origin, including (but not limited to), for example, mouse, rat, rabbbit, cat, dog, primate, and human. Preferably the nucleic acid provided by the invention encodes GalR2 receptors of mammalian, and most preferably, rat or human origin.

The invention also includes nucleotide sequences encoding chimeric proteins comprised of parts of the GalR2 receptor and parts of other related seven-transmembrane receptors.

The BMB77 clone (SEQ ID NO: 1) (*see* Example 1, *infra*) has a 1.7-kb cDNA insert with a open reading frame from nucleotides 279 to 1394 that encodes a 372 amino acid protein (SEQ ID NO: 2). Hydrophobicity plot analysis using the PEPPLOT function of GCG (Genetics Computer Group, Madison, WI) shows that the GalR2 receptor has seven transmembrane-like domains, indicating it might be a G-protein-coupled receptor. GalR2 is 26 amino acids longer in length than GalR1, the only other published galanin receptor. This extra length of amino acids within GalR2 is due to an extended C-terminal tail sequence. However, the putative N-terminal extracellular domain of GalR2 is about 7 amino acids shorter than the corresponding region in GalR1. It is also important to note that the GalR2 sequence shows only 38% amino acid sequence identity to the GalR1 receptor.

The Y107 clone (SEQ ID NO: 3) (*see* Example 1, *infra*) has a 1.9-kb cDNA insert

with an open reading frame from nucleotides 17-384, and from nucleotides 874-1621. The sequence between nucleotides 384 and 874 contains multiple STOP codons in all three reading frames. Furthermore, the dinucleotides GT and AG at positions 385-386 and 872-873, respectively, fulfill the criteria for being a splice donor and acceptor site, respectively. Moreover, when the nucleotide sequences 17-384 and 874-1621 are joined together, an open reading frame is formed which has a cognate translation product nearly identical to that of clone BMB77 (SEQ ID NO: 4). Therefore, it is likely that the region between these two open reading frames is an intron.

There is a single nucleotide change between positions 963 in clone BMB77 (SEQ ID NO: 1) and 1190 in clone Y107 (SEQ ID NO: 3). This adenine to cytosine transversion results in a change from Ser^{229} in clone BMB77 (SEQ ID NO: 2) to Arg^{229} in clone Y107 (SEQ ID NO: 4). Amino acid 229 is located in the third intracellular loop of GalR2. The third intracellular loop of other seven transmembrane domain G protein-coupled receptors is an important domain for effecting coupling of the receptor to its G protein. Bockaert, *Curr. Op. Neurobiol.* 1, 32-42 (1991). This polymorphic amino acid position could change the G protein binding characterisitics of the GalR2 receptor variants. Gillison, *Diabetes* 43, 24 (1994); Chen, *Am. J. Physiol.* 266, G113 (1994).

The partial human GalR2 nucleic acid sequence (SEQ ID NO: 5) contains a 337 amino acid opening reading frame. The initial leucine residue of this partial human GalR2 protein (SEQ ID NO: 6) corresponds to amino acid Leu^{51} in rat GalR2 (SEQ ID NO: 2 and 4).

Nucleic acid hybridization probes provided by the invention are DNAs consisting essentially of the nucleotide sequences complementary to any sequence depicted in SEQ ID NO:s 1 and 3 that is effective in nucleic acid hybridization. Nucleic acid probes are useful for detecting GalR2 gene expression in cells and tissues using techniques well-known in the art, including, but not limited to, Northern blot hybridization, *in situ* hybridization, and Southern hybridization to reverse transcriptase - polymerase chain reaction product DNAs. The probes provided by the present invention, including oligonucleotide probes derived therefrom, are also useful for Southern hybridization of mammalian, preferably human, genomic DNA for screening for restriction fragment length polymorphism (RFLP) associated with certain genetic disorders. As used herein, the term complementary means a nucleic acid having a sequence that is sufficiently complementary in the Watson-Crick sense to a target nucleic acid to bind to the target under physiological conditions or experimental conditions which those skilled in the art routinely use when employing probes.

Receptor GalR2 binds various fragments and analogs of galanin with affinities different from that of the known receptors. The rank order of binding affinity of receptor GalR2 was found to be:

galanin = (2-29)galanin > (1-15)galanin >> (3-29)galanin

Table 1, *infra*, presents a more detailed affinity profile of the GalR2 receptor for galanin and various fragments thereof. As used herein, a protein having substantially the same affinity profile as the GalR2 receptor means a protein in which the IC_{50} of each of the peptides listed in Table 1, *infra*, is no more than an order of magnitude greater than those listed in Table 1 for each of the respective peptides as measured according to the methods described in Example 2.

The production of proteins such as receptor GalR2 from cloned genes by genetic engineering means is well known in this art. The discussion which follows is accordingly intended as an overview of this field, and is not intended to reflect the full state of the art.

DNA which encodes receptor GalR2 may be obtained, in view of the instant disclosure, by chemical synthesis, by screening reverse transcripts of mRNA from appropriate cells or cell line cultures, by screening genomic libraries from appropriate cells, or by combinations of these procedures, as illustrated below. Screening of mRNA or genomic DNA may be carried out with oligonucleotide probes generated from the GalR2 gene sequence information provided herein. Probes may be labeled with a detectable group such as a fluorescent group, a radioactive atom or a chemiluminescent group in accordance with known procedures and used in conventional hybridization assays, as described in greater detail in the Examples below. In the alternative, the GalR2 gene sequence may be obtained by use of the polymerase chain reaction (PCR) procedure, with the PCR oligonucleotide primers being produced from the GalR2 gene sequence provided herein. *See* U.S. Patent Nos. 4,683,195 to Mullis *et al.* and 4,683,202 to Mullis.

Receptor GalR2 may be synthesized in host cells transformed with a recombinant expression construct comprising a nucleic acid encoding the receptor GalR2. Such a recombinant expression construct can also be comprised of a vector that is a replicable DNA construct. Vectors are used herein either to amplify DNA encoding GalR2 and/or to express DNA which encodes GalR2. For the purposes of this invention, a recombinant expression construct is a replicable DNA construct in which a DNA sequence encoding GalR2 is operably linked to suitable control sequences capable of effecting the expression of GalR2 in

a suitable host. The need for such control sequences will vary depending upon the host selected and the transformation method chosen. Generally, control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences which control the termination of transcription and translation. Amplification vectors do not require expression control domains. All that is needed is the ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants. *See*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (2nd Edition, Cold Spring Harbor Press, New York, 1989).

Vectors useful for practicing the present invention include plasmids, viruses (including phage), retroviruses, and integratable DNA fragments (*i.e.*, fragments integratable into the host genome by homologous recombination). The vector replicates and functions independently of the host genome, or may, in some instances, integrate into the genome itself. Suitable vectors will contain replicon and control sequences which are derived from species compatible with the intended expression host. The vectors may be self-replicating. Suitable vectors for the purposes of the present invention include pBluescript, pcDNA3, pSV-SPORT, and, for insect cells, baculovirus. A preferred vector is the plasmid pcDNA3 (Invitrogen, San Diego, CA).

Construction of suitable vectors containing the desired coding and control sequences employs standard ligation and restriction techniques that are well understood in the art. Isolated plasmids, DNA sequences, or synthesized oligonucleotides are cleaved, tailored, and relegated in the form desired.

Site-specific DNA cleavage is performed by treating with the suitable restriction enzyme (or enzymes) under conditions that are generally understood in the art, and the particulars of which are specified by the manufacturer of these commercially available restriction enzymes. *See, e.g., New England Biolabs, Product Catalog.* In general, about 1 μg of plasmid or DNA sequence is cleaved by one unit of enzyme in about 20 μl of buffer solution. Often excess of restriction enzyme is used to ensure complete digestion of the DNA substrate. Incubation times of about one hour to two hours at about 37°C are workable, although variations are tolerable. After each incubation, protein is removed by extraction with phenol/chloroform, and may be followed by ether extraction. The nucleic acid may be recovered from aqueous fractions by precipitation with ethanol. If desired, size separation of the cleaved fragments may be performed by polyacrylamide gel or agarose gel

electrophoresis using standard techniques. A general description of size separations is found in *Methods in Enzymology* 65, 499-560 (1980).

Transformed host cells are cells which have been transformed or transfected with recombinant expression constructs made using recombinant DNA techniques and comprising mammalian GalR2-encoding sequences. Preferred host cells for transient transfection are COS-7 cells. Transformed host cells may ordinarily express GalR2, but host cells transformed for purposes of cloning or amplifying nucleic acid hybridization probe DNA need not express the receptor. When expressed, the mammalian GalR2 protein will typically be located in the host cell membrane. *See*, Sambrook *et al.*, *ibid.*

Cultures of cells derived from multicellular organisms are desirable hosts for recombinant GalR2 protein synthesis. In principal, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. However, mammalian cells are preferred, as illustrated in the Examples. Propagation of such cells in cell culture has become a routine procedure. *See* Tissue Culture (Academic Press, Kruse & Patterson, Eds., 1973). Examples of useful host cell lines are bacteria cells, insect cells, yeast cells, human 293 cells, VERO and HeLa cells, LMTK$^-$ cells, and WI138, BHK, CHO, COS-7, CV, and MDCK cell lines (American Type Culture Collection, Rockville, MD). CHO cells are preferred.

The invention provides homogeneous compositions of mammalian GalR2 produced by transformed eukaryotic cells as provided herein. Such homogeneous compositions are intended to be comprised of mammalian GalR2 protein that comprises at least 90% of the protein in such homogenous composition. The invention also provides membrane preparation from cells expressing GalR2 as the result of transformation with a recombinant expression construct, as described here.

Mammalian GalR2 protein made from cloned genes in accordance with the present invention may be used for screening compounds for GalR2 agonist or antagonist activity, or for determining the amount of a GalR2 agonist or antagonist drug in a solution (*e.g.*, blood plasma or serum). For example, host cells may be transformed with a recombinant expression construct of the present invention, GalR2 protein expressed in those host cells, the cells lysed, and the membranes from those cells used to screen compounds for GalR2 binding activity. Competitive binding assays in which such procedures may be carried out are well known in the art. By selection of host cells which do not ordinarily express GalR2, pure or crude preparations of membranes containing GalR2 can be obtained. Further, GalR2 agonists and antagonists can be identified by transforming host cells with a recombinant expression

construct as provided by the present invention. Membranes obtained from such cells (and membranes of intact cells) can be used in binding studies wherein the drug dissociation activity is monitored.

It is known that the neurotransmitter galanin is a regulator of appetite, cognition, endocrine function, pain, and smooth muscle control. As shown herein, the various galanin analogs/fragments that induce these physiological responses bind with a high affinity to the GalR2 receptor. It is therefore evident that by modulating the activity of the GalR2 receptor, various physiological activities can be regulated. Specifically, antagonists to the GalR2 receptor, identified by the methods described herein, could be used to treat obesity, diabetes, hyperlipidemia, stroke, neuropathic pain, Alzheimer's disease, and/or endocrine disorders.

This invention provides a pharmaceutical composition comprising an effective amount of a drug identified by the method described herein and a pharmaceutically acceptable carrier. Such drugs and carrier can be administered by various routes, for example oral, subcutaneous, intramuscular, intravenous or intracerebral. The preferred route of administration would be oral at daily doses of about 0.01-100 mg/kg.

This invention provides a method of treating obesity, diabetes, hyperlipidemia, stroke, neuropathic pain, Alzheimer's disease, or endocrine disorders wherein the abnormality is improved by reducing the activity of GalR2 receptor or blocking the binding of ligands to a GalR2 receptor which comprises administering an effective amount of the pharmaceutical composition described above.

The recombinant expression constructs of the present invention are useful in molecular biology to transform cells which do not ordinarily express GalR2 to thereafter express this receptor. Such cells are useful as intermediates for making cell membrane preparations useful for receptor binding assays, which are in turn useful for drug screening. Drugs identified from such receptor assays can be used for the treatment of obesity, diabetes, anorexia, hyperlipidemia, stroke, neuropathic pain, Alzheimer's disease, or endocrine disorders.

The recombinant expression constructs of the present invention are also useful in gene therapy. Cloned genes of the present invention, or fragments thereof, may also be used in gene therapy carried out by homologous recombination or site-directed mutagenesis. *See generally* Thomas & Capecchi, *Cell* **51**, 503-512 (1987); Bertling, *Bioscience Reports* 7, 107-112 (1987); Smithies *et al.*, *Nature* **317**, 230-234 (1985).

Oligonucleotides of the present invention are useful as diagnostic tools for probing

GalR2 gene expression in tissues. For example, tissues are probed *in situ* with oligonucleotide probes carrying detectable groups by conventional autoradiographic techniques, as explained in greater detail in the Examples below, to investigate native expression of this receptor or pathological conditions relating thereto. Further, chromosomes can be probed to investigate the presence or absence of the GalR2 gene, and potential pathological conditions related thereto, as also illustrated by the Examples below. Probes according to the invention should generally be at least about 15 nucleotides in length to prevent binding to random sequences, but, under the appropriate circumstances may be smaller.

The invention also provides antibodies that are immunologically reactive to a mammalian GalR2, preferably rat or human GalR2. The antibodies provided by the invention are raised in animals by inoculation with cells that express a mammalian GalR2 or epitopes thereof, using methods well known in the art. Animals that are used for such inoculations include individuals from species comprising cows, sheep, pigs, mice, rats, rabbits, hamsters, goats and primates. Preferred animals for inoculation are rodents (including mice, rats, hamsters) and rabbits. The most preferred animal is the mouse.

Cells that can be used for such inoculations, or for any of the other means used in the invention, include any cell line which naturally expresses a mammalian GalR2, or any cell or cell line that expresses a mammalian GalR2 or any epitope thereof as a result of molecular or genetic engineering, or that has been treated to increase the expression of a mammalian GalR2 by physical, biochemical or genetic means. Preferred cells are human cells, most preferably HEK 293 cells that have been transformed with a recombinant expression construct comprising a nucleic acid encoding a mammalian GalR2, preferably a rat or human GalR2, and that express the mammalian GalR2 gene product.

The present invention provides monoclonal antibodies that are immunologically reactive with an epitope of mammalian GalR2 or fragment thereof and that is present on the surface of mammalian cells, preferably human or mouse cells. These antibodies are made using methods and techniques well known to those of skill in the art.

Monoclonal antibodies provided by the present invention are produced by hybridoma cell lines, that are also provided by the invention and that are made by methods well known in the art. Hybridoma cell lines are made by fusing individual cells of a myeloma cell line with spleen cells derived from animals immunized with cells expressing the GalR2 receptor, preferably rat or human cells, as described above. The myeloma cell lines used in the

invention include lines derived from myelomas of mice, rats, hamsters, primates and humans. Preferred myeloma cell lines are from mouse. The animals from whom spleens are obtained after immunization are rats, mice and hamsters, preferably mice, most preferably Balb/c mice. Spleen cells and myeloma cells are fused using a number of methods well known in the art, including but not limited to incubation with inactivated Sendai virus and incubation in the presence of polyethylene glycol (PEG). The most preferred method for cell fusion is incubation in the presence of a solution of 45% (w/v) PEG-1450. Monoclonal antibodies produced by hybridoma cell lines can be harvested from cell culture supernatant fluids from *in vitro* cell growth; alternatively, hybridoma cells can be injected subcutaneously and/or into the peritoneal cavity of an animal, most preferably a mouse, and the monoclonal antibodies obtained from blood and/or ascites fluid.

Monoclonal antibodies provided by the present invention are also produced by recombinant genetic methods well known to those of skill in the art, and the present invention encompasses antibodies made by such methods that are immunologically reactive with an epitope of a mammalian GalR2.

The present invention encompasses fragments of the antibody that are immunologically reactive with an epitope of a mammalian GalR2. Such fragments are produced by any number of methods, including but not limited to proteolytic cleavage, chemical synthesis or preparation of such fragments by means of genetic engineering technology. The present invention also encompasses single-chain antibodies that are immunologically reactive with an epitope of a mammalian GalR2 made by methods known to those of skill in the art.

The present invention also encompasses an epitope of a mammalian GalR2 that is comprised of sequences and/or a conformation of sequences present in the mammalian GalR2 molecule. This epitope may be naturally occurring, or may be the result of proteolytic cleavage of the mammalian GalR2 molecule and isolation of an epitope-containing peptide or may be obtained by synthesis of an epitope-containing peptide using methods well known to those skilled in the art. The present invention also encompasses epitope peptides produced as a result of genetic engineering technology and synthesized by genetically engineered prokaryotic or eukaryotic cells.

The invention also includes chimeric antibodies, comprised of light chain and heavy chain peptides immunologically reactive to an epitope that is a mammalian GalR2. The chimeric antibodies embodied in the present invention include those that are derived from

naturally occurring antibodies as well as chimeric antibodies made by means of genetic engineering technology well known to those of skill in the art.

Also provided by the present invention are non-human transgenic animals grown from germ cells transformed with the GalR2 nucleic acid sequence according to the invention and that express the GalR2 receptor according to the invention and offspring and descendants thereof. Also provided are transgenic non-human mammals comprising a homologous recombination knockout of the native GalR2 receptor, as well as transgenic non-human mammals grown from germ cells transformed with nucleic acid antisense to the GalR2 nucleic acid of the invention and offspring and descendants thereof. Further included as part of the present invention are transgenic animals which the native GalR2 receptor has been replaced with the human homolog. Of course, offspring and descendants of all of the foregoing transgenic animals are also encompassed by the invention.

Transgenic animals according to the invention can be made using well known techniques with the nucleic acids disclosed herein. *E.g.*, Leder et al., U.S. Patent Nos. 4,736,866 and 5,175,383; Hogan et al., *Manipulating the Mouse Embryo, A Laboratory Manual* (Cold Spring Harbor Laboratory (1986)); Capecchi, *Science* **244**, 1288 (1989); and Zimmer and Gruss, *Nature* **338**, 150 (1989). Such transgenic animals are useful for screening for and determining the physiological effects of GalR2 receptor agonists and antogonist. Consequently, such transgenic animals are useful for developing drugs to regulate physiological activities in which galanin participates.

The following Examples are provided for illustrative purposes only and are not intended, nor should they be construed, as limiting the invention in any manner.

EXAMPLES

Example 1: Isolation and Sequencing of Rat GalR2 Receptor

Isolation of rat hypothalamus mRNA and construction of cDNA library

An expression cloning strategy was used to clone the novel galanin receptor from a rat hypothalamus cDNA library. RNA was obtained from 9 frozen rat hypothalami weighing a total of 0.87 grams. Poly(A) RNA was isolated directly from the tissue using the Promega PolyATtract System 1000 kit (Promega (Madison, WI) Z5420). The hypothalami were homogenized in 4 mL of 4M guanidine thiocyanate-25mM sodium citrate, pH 7.1-2% ß-mercaptoethanol using a Polytron at full-speed for approximately 1 minute. To the homogenized tissue 8 mL of 4M guanidine thiocyanate-25mM sodium citrate, pH 7.1-1% ß-

mercaptoethanol which had been preheated to 70°C was added. After mixing thoroughly, 870 pmol biotinylated oligo(dT) was added; the mixture was incubated at 70°C for 5 minutes. The homogenate was subjected to centrifugation at 12000 x *g* for 10 minutes at room temperature; the homogenate was transferred to a clean tube and 10.44 mL Streptavidin MAGNESPHERE® Paramagnetic Particles (SA-PMPs) which had been prepared as per the published protocol was added. (Promega Corporation (Madison, WI) published protocol TM 228). The homogenate and SA-PMPs were incubated together for 2 minutes at room temperature after which the homogenate was decanted while the SA-PMP-biotinylated oligo(dT)-hypothalamic poly(A) RNA complex was retained in the tube by a magnetic stand. The complex was washed as per the protocol, after which the RNA was precipitated and resuspended in water. 25 micrograms of this poly(A) RNA was used by Invitrogen (Invitrogen Corporation, San Diego, CA) to prepare a cDNA expression library. The protocols used by Invitrogen to prepare the cDNA library are essentially based upon the procedures of Okayama and Berg (*Molec. Cell. Biol.* 2, 161 (1982)) and Gubler and Hoffman (*Gene* 25, 263 (1983)) (Invitrogen Corporation (San Diego, CA) publications 130813sa and 130928sa). An oligo(dT) anchor primer was used for reverse transcription, and the library was cloned unidirectionally into pcDNA3 vector which contains a CMV (cytomegalovirus) promoter for eukaryotic expression. The cDNA library had 5.3 x 10^5 primary recombinants with an average insert size of 2.59 kb.

Isolation of a novel galanin receptor cDNA clone

1. Homology cloning strategy

In order to isolate novel receptor(s) for galanin, approximately 2 million phage plaques of a rat small intestine library (Stratagene (La Jolla, CA) 936508) were screened with rat GalR1 coding sequence DNA as probe under low stringency conditions (30% formamide, 6X SSC (0.9M NaCl/0.09M NaCitrate), 0.1% N-lauroyl sarcosine, 0.2% SDS, 3% blocking reagent.) The probe was prepared by digesting the parent GalR1 plasmid with SacI and AccI, separating the fragments by agarose gel electrophoresis, and purifying the 1-kb SacI-AccI fragment from the gel. The probe was labeled with digoxigenin dUTP according to the manufacturer's instructions (GENIUS Kit, Boehringer Mannheim, Indianapolis, IN, PN 1093 657). The filter lifts, denaturation, neutralization, hybridization, and washing were done according to the manufacturer's instructions except that hybridization was done at 30°C and the washes were performed twice for 40 minutes each: once at room temperature; the second

at 37°C.

One plaque containing DNA homologous to the probe was purified and subcloned into pBluescript vector (Stratagene (La Jolla, California) 212206) by standard molecular biological techniques. This clone, designated SI2112, was subjected to sequence analysis and was found to contain a sequence which had characteristics of a novel, but truncated member of the G-protein-coupled 7TMD receptor family. This clone was later used as probe to determine the identity of a novel galanin-binding clone (Y107) found in the expression cloning strategy (see below.)

The partial human GalR2 cDNA was obtained by screening approximately two million phage of a human small intestine library (Clontech (Palo Alto, CA) HL 1133a) were screened with a human GalR2 probe obtained by PCR amplification from human genomic DNA. The conditions used were essentially as described for the rat GalR2 isolation.

2. Expression cloning strategy

The rat hypothalamus cDNA library was plated on the Luria Broth/Ampicillin (GIBCO) plates in pools of 1,000 independent colonies. The plates were incubated at 37°C for about 20 hours and the bacteria from each plate were scraped in 4-5 ml LB/Ampicillin media. Two ml of the bacteria samples were used for plasmid preparation and one ml of each pool was stored at -80°C in 15% glycerol.

COS-7 cells were grown in Dulbecco's Modified Eagle Medium (DMEM, GIBCO (Gaithersburg, MD) 11965-092), 10% fetal bovine serum (GIBCO (Gaithersburg, MD) 16000-028), and 1X antibiotic/antimycotic solution (GIBCO (Gaithersburg, MD) 15240-039). Cells were maintained by trypsinizing and splitting at 50 to 70% confluency.

Twenty-four hours before transfection, cells were plated into flaskette chambers (Nunc, Inc. (Naperville, IL) 177453) at $3x10^5$ cells/flaskette (equivalent to $3x10^4$ cells/cm^2). Two μg of plasmid DNA from each pool was transfected into the cells using 10 μl of Lipofectamine (GIBCO (Gaithersburg, MD) 18324-012) according to the manufacturer's protocol.

Forty-eight hours after transfection, the [^{125}I]galanin binding assay was performed in the flaskette chamber. The cells were washed once with 25 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.4, and blocked for 15 minutes with 1 ml total binding buffer (25 mM Tris-HCl, 10 mM $MgCl_2$, 1% bovine serum albumin, pH 7.4) at room temperature. After aspirating off the blocking solution, 1 ml of binding buffer containing 100 pM ^{125}I-hGalanin (NEN DuPont

(Boston, MA) NEX-333) was added and flasks were incubated at room temperature for 90 minutes. Following the incubation, the labeling buffer was removed and the flaskettes were rinsed (approximately 2 mL per flaskette) four times with ice-cold binding buffer. After a final rinse with ice-cold phosphate buffered saline (PBS)(GIBCO (Gaithersburg, MD) 14190-136), the cells were fixed with ice-cold PBS/1% glutaraldehyde (Sigma (St. Louis, MO) G5882). The solution was removed and residual glutaraldehyde rinsed away with one wash of ice-cold PBS/0.5 M Tris (pH 7.5) followed by one wash of ice-cold PBS. After separating the slide bases from their tops, the slides were dipped in 0.5% gelatin at 42°C and dried under vacuum. The dried slides were dipped in photographic emulsion (NTB-2) (Kodak (Rochester, NY) 165 4433) diluted 1:1 in 0.02% Aerosol-OT (Sigma (St. Louis, MO) A6627) at 42°C, dried at room temperature for 1 hour, and exposed in the darkbox for four or five days at 4°C. After sufficient exposure time, the darkbox was brought to room temperature for 1 hour after which the slides were developed in D-19 developer (Kodak (Rochester, NY) 146 4593) for three minutes at 15°C, placed in fixer (Kodak (Rochester, NY) 197 1746) for three minutes at 15°C, washed in water, and air dried. Cells were stained with Diff-Quik stain set (Baxter (McGaw Park, IL) B4132-1) and air dried. Slides were dipped into xylenes and mounted with DPX mountant (Electron Microscopy Sciences (Fort Washington, PA) 13510). Positive cells were identified using dark field microscopy.

Two positive pools were identified. Since the hypothalamus could express different subtypes of galanin receptor, we analyzed the positive pools for GalR1 receptors by PCR and homology. Of the 2 positive pools tested as described above, 1 contained GalR1 as determined by PCR and homology analyses. However, the other pool (Y107) was negative for GalR1 by PCR, but homologous to SI2112 probe (see *Homology* strategy, above). Because: 1) SI2112 sequence indicated it was likely a novel, albeit truncated and unexpressible, G-protein-coupled receptor; 2) pool Y107 DNA showed homology to the SI2112 probe; and, 3) DNA from pool Y107, when used to transiently transfect COS-7 cells, conferred the ability upon the cells to bind galanin, it was deduced that the clone within pool Y107, which conferred galanin-binding ability when expressed, was likely to be an expressible version of the SI2112 clone. Therefore, clones from pool Y107 were probed with radiolabeled DNA prepared from SI2112, and a single clone hybridizing to the SI2112 probe was purified from non-homologous clones. This clone was called Y107.

Sequence analysis of clone Y107 (SEQ ID NO: 3) revealed the presence of one intron of 489-bp length, beginning after nucleotide 384 (the second nucleotide of the codon for

amino acid 133, an arginine residue). Using Y107 DNA as probe, an intronless version of the Y107 cDNA was obtained from a PC12 cell cDNA library by homology cloning. The first intronless version of the Y107 was in the pSV-SPORT vector (GIBCO (Gaithersburg, MD) 15386-014); the complete cDNA insert of this clone was subcloned into the pcDNA3 vector (Invitrogen (San Diego, CA) V790-20) in order to maintain, for subsequent pharamacological analyses, common vector and promoter (CMV) backgrounds amongst our clones. The intronless clone in pSV-SPORT was designated BMB77.sv40; the intronless clone contained within pcDNA3 is named BMB77. Clones BMB77 and Y107 differ by one amino acid in sequence. Pharmacological analyses have been performed with both the Y107 (SEQ ID NO: 3 and 4) and BMB77 (SEQ ID NO: 1 and 2) clones.

DNA and peptide sequences analysis

Plasmid DNA was sequenced by Lark Technologies Inc. (Houston, Texas) and Biotechnology Resource Laboratory of Yale University (New Haven, CT) using Sequenase Kit (US Biochemical (Cleveland, OH) 70770) or Applied Biosystems' automatic sequencer system (Model 373A). The peptide sequence was deduced from the long open-reading-frame of the nucleotide sequence. DNA and peptide sequences were analyzed using the GCG program (Genetics Computer Group, Madison, WI). The results are embodied in SEQ ID NO: 1 (the nucleic acid sequence of clone BMB77), SEQ ID NO: 2 (the amino acid sequence of clone BMB77), SEQ ID NO: 3 (the nucleic acid sequence of clone Y107), SEQ ID NO: 4 (the amino acid sequence, omitting the putative intronic region, of clone Y107), SEQ ID NO: 5 (the partial nucleic acid sequence of human GalR2), and SEQ ID NO: 6 (the partial amino acid sequence of human GalR2).

Example 2: Pharmacological Characterization of the Novel Rat Galanin Receptor

Transient Transfection

Monkey kidney cells (COS-7) were maintained in T-175 cm^2 flasks (Nunc, Inc. (Naperville, IL) 171226) at 37°C with 5% CO_2 in a humidified atmosphere. Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) (GIBCO (Gaithersburg, MD) 11965-092) supplemented with 2 mM glutamine, 10% fetal bovine serum, 1 mM sodium pyruvate and a antibiotic/antimycotic comprised of pennicillin/streptomycin/amphotericinB (GIBCO, Gaithersburg, MD, PN 15240-013). Cells at 70% confluency were transfected with rat GalR2 DNA using the Lipofectamine reagent (GIBCO (Gaithersburg, MD) 18324-012). 15 μg of

DNA and 90 µl of lipofectamine were added to each flask. Media was replaced 24 hours post transfection, and membranes were harvested 24 hours later.

Stable Expression of the Rat GalR2 Receptor (clone BMB77)

293 cells (human embryonic kidney, ATCC) were plated onto a T-25 flask one day prior to transfection, such that they were 50-70% confluent at the time of transfection. 15 ug of rat GalR2 (BMB77) DNA were added to 0.3 ml of Optimem I culture media (GIBCO Life Sciences), and 25 ul of lipofectamine were added to 0.3 ml of Optimem I. The DNA and lipofectamine solutions were combined and incubated at room temperature for 20 minutes. An additional 2.4 ml of Optimem I was added to the DNA/lipofectamine mixture. The media was aspirated from T-25 flask containing 293 cells, washed with Optimem I, and the DNA/lipofectamine mixture was added to the 293 cells. After a 5-6 hour incubation in a 37°C incubator (5% CO_2) for 5-6 hours, the DNA/lipofectamine mixture was replaced with culture media (DMEM, 10% fetal bovine serum, 2 mM glutamine and antibiotic/antimycotic.)

The day following transfection, the media was replaced with selecting media (culture media with the addition of 350 ug/ml of Geneticin G-418), and the flask was returned to the 37°C / 5% CO_2 incubator. When discrete colonies became apparent, cells were pooled. Growth was monitored, followed by cloning by limited dilution, such that an average of one cell was seeded in each well of a 96-well microtiter culture plate. After about 21 days in culture under selection conditions, those wells containing single colonies were selected and transferred to 24-well culture plates pretreated with poly-l-lysine, following trypsinization. Each of these clones was propagated until sufficient quantities were available for testing in the [^{125} I]galanin binding assay, from which one particular clone designated 293-rGalR2-1 was selected on the basis of its high level of receptor expression.

Membrane Preparation

The media was removed from each flask of transfected cells, and the cells were washed twice with 20 ml ice-cold phosphate buffered saline. The cells were scraped from the flask in 5 ml of Tris buffer (20 mM Tris-HCl, 5 mM EDTA, pH 7.7), and then transferred to a centrifuge tube. Each flask was rinsed with an additional 5 ml of Tris buffer, and combined in the centrifuge tube. The cells were homogenized in a Polytron PT-3000 (Brinkman Instruments, Mill Valley, New York) for 2 x 10 seconds (12 mM probe, 7000-8000 rpm) and

centrifuged at 20,000 x g for 30 minutes at 4°C. The pellet was resuspended in fresh Tris buffer, and centrifuged again at 20,000 x g for 30 minutes at 4°C. Protein concentration was measured using the Bio-Rad kit according to the standard manufacturer's protocol (Bio-Rad Laboratories (Hercules, CA) 500-0001) using bovine IgG as the standard.

[^{125}I]Galanin Binding Assay for rat GalR1 and rat GalR2 clones

The binding assays were performed on 96-well plates (GF/C Millipore Corp., Bedford, MA PN MAFC NOB 50) pretreated with 0.3% polyethylenimine (PEI) for at least 3 hours prior to use. The PEI was aspirated from the plates on a vacuum manifold, and the wells were rinsed with 200 µl of ice-cold binding buffer (25 mM Tris, 10 mM $MgCl_2$, 0.1% BSA, pH 7.4) immediately before samples were added to the wells. For competition assays, increasing concentrations of peptide were incubated with [^{125}I]hGalanin (NEN DuPont (Boston, MA) NEX333) and membrane. In a final volume of 200 µl, samples consisted of ~1.25 µg of protein, 50 pM [^{125}I]hGalanin, and peptide dilution or binding buffer. Nonspecific binding was defined by 100 nM rat galanin. For saturation experiments, increasing concentrations of [^{125}I]hGalanin were incubated with membrane and 100 nM rat galanin. Samples were incubated for 90 minutes at room temperature with constant shaking. To terminate the reaction, samples were aspirated on a vacuum manifold and rinsed with 3 x 200 µl ice-cold buffer. Samples were then counted on a gamma counter to quantitate the amount of radioactivity. Rat galanin, fragment peptides (1-15)galanin, (1-12)galanin, (1-10)galanin, chimeric peptide M40, (2-29)rat galanin, (3-29)rat galanin, (5-29)rat galanin, (9-29)rat galanin, (10-29)rat galanin, (2-30)human galanin, and (3-30)human galanin were synthesized at Bayer Corp. (West Haven, CT). All other peptides were purchased from either Peninsula (Belmont, CA) or Bachem (Torrance, CA).

In Vitro Pharmacology

Saturation analysis for rat GalR2 transiently expressed in COS-7 cells yielded a one-component model with an average K_d value of 0.28 nM (n=2) and a receptor density (B_{max}) of 770 fmol/mg protein (Fig. 6). This is very similar to the rat GalR2 receptor stably expressed in 293 cells, which yielded an average K_d value of 0.20 nM (n=2) and a receptor density (B_{max}) of 2289 fmol/mg protein (data not shown).

Table 1 summarizes the IC_{50} values (50% inhibition of specific binding, as determined using nonlinear regression analysis) of various standard peptides, fragments and chimeras for

[^{125}I]hGalanin binding to membranes expressing the rGalR2 receptor. Transiently expressed rat GalR1 is included in Table 1 for comparison of its pharmacological profile to this novel rat GalR2 receptor. The preliminary pharmacological binding profile for rat GalR2 differs from rat GalR1 such that rat galanin itself has about 10-fold lower affinity for GalR2, no matter how it is expressed. When comparing transiently expressed GalR1 and GalR2, the chimeric peptide C7 also has about 10-fold lower affinity for GalR2; this difference, however, is not observed with rat GalR2 stably expressed in 293 cells. Rat(1-16)galanin has about 5- to 10-fold lower affinity for GalR2 than GalR1 (Table 1). In addition, the ratio of affinities of (1-12) and (1-15)galanin are markedly different for GalR1 (22-fold difference), while these two peptides have more equivalent binding affinities for GalR2.

The binding profiles for intron-containing (Y107) and intronless (BMB77 clone) GalR2 receptors transiently expressed are very similar, with the possible exceptions of M15 and (1-12)galanin, which have approximately 5- and 3-fold higher affinities, respectively, for clone Y107.

Table 1

PEPTIDE	BINDING AFFINITY - IC_{50} VALUES (nM)			
	Rat GalR2 [clone Y107 - transient]	Rat GalR2 [clone BMB77 - transient]	293-rGalR2-1 [clone BMB77 - stable]	Rat GalR1 [cloned - transient]
r(1-29)gal	0.46 (0.32,0.60)	0.58 ± 0.04	0.70 ± 0.05	0.061 ± 0.007
h(1-30)gal	0.29	0.45 (0.32,0.57)	ND	0.037(0.040,0.033)
r(2-29)gal	0.41	0.65 ± 0.14	0.77 ± 0.05	1.6 ± 0.42
M35	0.72	0.75 ± 0.04	1.2 ± 0.4	ND
M40	0.78 (0.75,0.81)	0.77 ± 0.03	1.2 ± 0.07	0.32 ± 0.10
r(1-16)gal	0.86 (0.97,0.75)	1.2 ± 0.16	1.1 ± 0.15	0.18 ± 0.02
h(2-30)gal	0.94	0.65 (0.8,0.5)	ND	1.9 ± 0.26
(1-15)gal	1.0 (1.1,0.99)	1.2 ± 0.21	2.4 ± 0.98	1.1 ± 0.27
(1-12)gal	1.6 (1.8,1.4)	5.7 ± 0.49	4.1 ± 1.0	24 (22,25)
C7	2.6	3.2 ± 0.56	0.44 ±0.06	0.26 ± 0.02
M15	7.0	38 ± 9.8	ND	2.0 (1.5,2.4)
(1-10)gal	240 (284,195)	393 ± 37	560 ± 89	ND
r(3-29)gal	>1000	>1000	>1000	>1000
h(3-30)gal	>1000	ND	ND	>1000
r(5-29)gal	>1000	>1000	ND	>1000
r(9-29)gal	>1000	>1000	ND	>1000
r(10-29)gal	>1000	>1000	ND	>1000

Table 1 summarizes the IC_{50} values for various standard peptides for [^{125}I]hGalanin binding to rat GalR1 and GalR2 clones. The averages ± standard error of the mean (SEM) represent values from at least three independent experiments. Two independent experiments are represented by the average, followed by the individual values in parentheses. Remaining values without SEM are from a single experiment. Peptide species are indicated with the following prefixes: r = rat, h = human. ND = not determined

Abbreviations of Chimers:

M15 = (1-13)Galanin + (5-11)Substance P = Galantide (see Bartfai, *TIPS* **13**, 312-317 (1992)

M35 = (1-13)Galanin + (2-9)Bradykinin (Bartfai, *infra*)

M40 = (1-13)Galanin + ProPro(AlaLeu)$_2$Ala amide (Bartfai, *infra*)

C7 = (1-13)Galanin + spantide (see Crawley, *Brain Research* **600**, 268-272 (1993)

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed with any wide range of equivalent parameters of composition, conditions, and methods of preparing such recombinant molecules, vectors, transformed hosts and proteins without departing from the spirit or scope of the invention or any embodiment thereof.

SEQ ID NO: 1: Clone BMB77 nucleic acid sequence

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   1 TCGACCCACG CGTCCGCTCA AGTCTAAAGC AGAGCGAGTC CCAGGACTTG
  51 AGCGCGGGAA GCGAATGGAG TCAGGGTCAT TCGATTGCAC CTCTCTCGGC
 101 TGCGGGCCGG AGCGGGGTAC CATCCTACAC TCTGGGTGCT CCCTCCTCCT
 151 CCCGTCCCCC GCGCACCCCT GCCCTGGCTC CTGGAGCTCG GCAGTCTCGC
 201 TGGGGCGCTG CAGCGAGGGA GCAGCGTGCT CACCAAGACC CGGACAGCTG
 251 CGGGAGCGGC GTCCACTTTG GTGATACCAT GAATGGCTCC GGCAGCCAGG
 301 GCGCGGAGAA CACGAGCCAG GAAGGCGGTA GCGGCGGCTG GCAGCCTGAG
 351 GCGGTCCTTG TACCCCTATT TTTCGCGCTC ATCTTCCTCG TGGGCACCGT
 401 GGGCAACGCG CTGGTGCTGG CGGTGCTGCT GCGCGGCGGC CAGGCGGTCA
 451 GCACCACCAA CCTGTTCATC CTCAACCTGG GCGTGGCCGA CCTGTGTTTC
 501 ATCCTGTGCT GCGTGCCTTT CCAGGCCACC ATCTACACCC TGGACGACTG
 551 GGTGTTCGGC TCGCTGCTCT GCAAGGCTGT TCATTTCCTC ATCTTTCTCA
 601 CTATGCACGC CAGCAGCTTC ACGCTGGCCG CCGTCTCCCT GGACAGGTAT
 651 CTGGCCATCC GCTACCCGCT GCACTCCCGA GAGTTGCGCA CACCTCGAAA
 701 CGCGCTGGCC GCCATCGGGC TCATCTGGGG GCTAGCACTG CTCTTCTCCG
 751 GGCCCTACCT GAGCTACTAC CGTCAGTCGC AGCTGGCCAA CCTGACAGTA
 801 TGCCACCCAG CATGGAGCGC ACCTCGACGT CGAGCCATGG ACCTCTGCAC
 851 CTTCGTCTTT AGCTACCTGC TGCCAGTGCT AGTCCTCAGT CTGACCTATG
 901 CGCGTACCCT GCGCTACCTC TGGCGCACAG TCGACCCGGT GACTGCAGGC
 951 TCAGGTTCCC AGAGCGCCAA ACGCAAGGTG ACACGGATGA TCATCATCGT
1001 GGCGGTGCTT TTCTGCCTCT GTTGGATGCC CCACCACGCG CTTATCCTCT
1051 GCGTGTGGTT TGGTCGCTTC CCGCTCACGC GTGCCACTTA CGCGTTGCGC
1101 ATCCTTTCAC ACCTAGTTTC CTATGCCAAC TCCTGTGTCA ACCCCATCGT
1151 TTACGCTCTG GTCTCCAAGC ATTTCCGTAA AGGTTTCCGC AAAATCTGCG
1201 CGGGCCTGCT GCGCCCTGCC CCGAGGCGAG CTTCGGGCCG AGTGAGCATC
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1251 CTGGCGCCTG GGAACCATAG TGGCAGCATG CTGGAACAGG AATCCACAGA

1301 CCTGACACAG GTGAGCGAGG CAGCCGGGCC CCTTGTCCCA CCACCCGCAC

1351 TTCCCAACTG CACAGCCTCG AGTAGAACCC TGGATCCGGC TTGTTAAAGG

1401 ACCAAAGGGC ATCTAACAGC TTCTAGACAG TGTGGCCCGA GGATCCCTGG

1451 GGGTTATGCT TGAACGTTAC AGGGTTGAGG CTAAAGACTG AGGATTGATT

1501 GTAGGGAACC TCCAGTTATT AAACGGTGCG GATTGCTAGA GGGTGGCATA

1551 GTCCTTCAAT CCTGGCACCC GAAAAGCAGA TGCAGGAGCA GGAGCAGGAG

1601 CAAAGCCAGC CATGGAGTTT GAGGCCTGCT TGAACTACCT GAGATCCAAT

1651 AATAAAACAT TTCATATGCT GTGAAAAAAA AAAAAAAAAA

SEQ ID NO: 2: Clone BMB77 amino acid sequence

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1    ATG AAT GGC TCC GGC AGC CAG GGC GCG GAG AAC ACG AGC CAG GAA GGC GGT AGC GGC GGC
1    Met Asn Gly Ser Gly Ser Gln Gly Ala Glu Asn Thr Ser Gln Glu Gly Gly Ser Gly Gly

61   TGG CAG CCT GAG GCG GTC CTT GTA CCC CTA TTT TTC GCG CTC ATC TTC CTC GTG GGC ACC
21   Trp Gln Pro Glu Ala Val Leu Val Pro Leu Phe Phe Ala Leu Ile Phe Leu Val Gly Thr

121  GTG GGC AAC GCG CTG GTG CTG GCG GTG CTG CTG CGC GGC GGC CAG GCG GTC AGC ACC ACC
41   Val Gly Asn Ala Leu Val Leu Ala Val Leu Leu Arg Gly Gly Gln Ala Val Ser Thr Thr

181  AAC CTG TTC ATC CTC AAC CTG GGC GTG GCC GAC CTG TGT TTC ATC CTG TGC TGC GTG CCT
61   Asn Leu Phe Ile Leu Asn Leu Gly Val Ala Asp Leu Cys Phe Ile Leu Cys Cys Val Pro

241  TTC CAG GCC ACC ATC TAC ACC CTG GAC GAC TGG GTG TTC GGC TCG CTG CTC TGC AAG GCT
81   Phe Gln Ala Thr Ile Tyr Thr Leu Asp Asp Trp Val Phe Gly Ser Leu Leu Cys Lys Ala

301  GTT CAT TTC CTC ATC TTT CTC ACT ATG CAC GCC AGC AGC TTC ACG CTG GCC GCC GTC TCC
101  Val His Phe Leu Ile Phe Leu Thr Met His Ala Ser Ser Phe Thr Leu Ala Ala Val Ser

361  CTG GAC AGG TAT CTG GCC ATC CGC TAC CCG CTG CAC TCC CGA GAG TTG CGC ACA CCT CGA
121  Leu Asp Arg Tyr Leu Ala Ile Arg Tyr Pro Leu His Ser Arg Glu Leu Arg Thr Pro Arg

421  AAC GCG CTG GCC GCC ATC GGG CTC ATC TGG GGG CTA GCA CTG CTC TTC TCC GGG CCC TAC
141  Asn Ala Leu Ala Ala Ile Gly Leu Ile Trp Gly Leu Ala Leu Leu Phe Ser Gly Pro Tyr

481  CTG AGC TAC TAC CGT CAG TCG CAG CTG GCC AAC CTG ACA GTA TGC CAC CCA GCA TGG AGC
161  Leu Ser Tyr Tyr Arg Gln Ser Gln Leu Ala Asn Leu Thr Val Cys His Pro Ala Trp Ser

541  GCA CCT CGA CGT CGA GCC ATG GAC CTC TGC ACC TTC GTC TTT AGC TAC CTG CTG CCA GTG
181  Ala Pro Arg Arg Arg Ala Met Asp Leu Cys Thr Phe Val Phe Ser Tyr Leu Leu Pro Val

601  CTA GTC CTC AGT CTG ACC TAT GCG CGT ACC CTG CGC TAC CTC TGG CGC ACA GTC GAC CCG
201  Leu Val Leu Ser Leu Thr Tyr Ala Arg Thr Leu Arg Tyr Leu Trp Arg Thr Val Asp Pro

661  GTG ACT GCA GGC TCA GGT TCC CAG AGC GCC AAA CGC AAG GTG ACA CGG ATG ATC ATC ATC
221  Val Thr Ala Gly Ser Gly Ser Gln Ser Ala Lys Arg Lys Val Thr Arg Met Ile Ile Ile

721  GTG GCG GTG CTT TTC TGC CTC TGT TGG ATG CCC CAC CAC GCG CTT ATC CTC TGC GTG TGG
241  Val Ala Val Leu Phe Cys Leu Cys Trp Met Pro His His Ala Leu Ile Leu Cys Val Trp

781  TTT GGT CGC TTC CCG CTC ACG CGT GCC ACT TAC GCG TTG CGC ATC CTT TCA CAC CTA GTT
261  Phe Gly Arg Phe Pro Leu Thr Arg Ala Thr Tyr Ala Leu Arg Ile Leu Ser His Leu Val

841  TCC TAT GCC AAC TCC TGT GTC AAC CCC ATC GTT TAC GCT CTG GTC TCC AAG CAT TTC CGT
281  Ser Tyr Ala Asn Ser Cys Val Asn Pro Ile Val Tyr Ala Leu Val Ser Lys His Phe Arg

901  AAA GGT TTC CGC AAA ATC TGC GCG GGC CTG CTG CGC CCT GCC CCG AGG CGA GCT TCG GGC
301  Lys Gly Phe Arg Lys Ile Cys Ala Gly Leu Leu Arg Pro Ala Pro Arg Arg Ala Ser Gly
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961 CGA GTG AGC ATC CTG GCG CCT GGG AAC CAT AGT GGC AGC ATG CTG GAA CAG GAA TCC ACA
321 Arg Val Ser Ile Leu Ala Pro Gly Asn His Ser Gly Ser Met Leu Glu Gln Glu Ser Thr

1021 GAC CTG ACA CAG GTG AGC GAG GCA GCC GGG CCC CTT GTC CCA CCA CCC GCA CTT CCC AAC
341 Asp Leu Thr Gln Val Ser Glu Ala Ala Gly Pro Leu Val Pro Pro Pro Ala Leu Pro Asn

1081 TGC ACA GCC TCG AGT AGA ACC CTG GAT CCG GCT TGT TAA 1119
361 Cys Thr Ala Ser Ser Arg Thr Leu Asp Pro Ala Cys * 372

SEQ ID NO: 3: Clone Y107 nucleic acid sequence

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   1 CCACTTTGGT GATACCATGA ATGGCTCCGG CAGCCAGGGC GCGGAGAACA
  51 CGAGCCAGGA AGGCGGTAGC GGCGGCTGGC AGCCTGAGGC GGTCCTTGTA
 101 CCCCTATTTT TCGCGCTCAT CTTCCTCGTG GGCACCGTGG GCAACGCGCT
 151 GGTGCTGGCG GTGCTGCTGC GCGGCGGCCA GGCGGTCAGC ACCACCAACC
 201 TGTTCATCCT CAACCTGGGC GTGGCCGACC TGTGTTTCAT CCTGTGCTGC
 251 GTGCCTTTCC AGGCCACCAT CTACACCCTG GACGACTGGG TGTTCGGCTC
 301 GCTGCTCTGC AAGGCTGTTC ATTTCCTCAT CTTTCTCACT ATGCACGCCA
 351 GCAGCTTCAC GCTGGCCGCC GTCTCCCTGG ACAGGTAAAG GACCCAGAAA
 401 GAAACATCCA GTATGCCCGG AGGGATCTTG ACTGGAAAAG ACTGAATCCT
 451 GGTCTGGTGA CCTTAGTTCC CTGCCCTTTC ACATCACTTG GACATTCCCA
 501 CAGAAGAGCG GTGAAGAGGC GGTGGTCCTT ATTCTCCTCT GGTTTCCACT
 551 GAGTGCAACA TGTGCGTCCT GAGTACGCTG GAGGGACTCA CAAAATTTCA
 601 GCTTTCTTTA GGAGTTTCCT TGCTGTAGTT TGACCCAAGT CTTCTCCAGG
 651 TTTCTGTCAG AACTCAGGCA TGAGGGATCT GCCTCCCCTG GTTGTCACCA
 701 GAGGATAACA ATCACTGCCC CCAGAAATCC AGACAGATTC TACAACTTTT
 751 AGTCTTCGGT GTTTTGGGGG TGCCCCTTCA CGTGGAGTAG GTCGGTGGCC
 801 ACATTCCCAG GAGTGACAAT AGCCTAGCAG TGAATCCTCT CGCTTAGCTG
 851 ATGCCCCCCC ACTGTCCCCA CAGGTATCTG GCCATCCGCT ACCCGCTGCA
 901 CTCCCGAGAG TTGCGCACAC CTCGAAACGC GCTGGCCGCC ATCGGGCTCA
 951 TCTGGGGGCT AGCACTGCTC TTCTCCGGGC CCTACCTGAG CTACTACCGT
1001 CAGTCGCAGC TGGCCAACCT GACAGTATGC CACCCAGCAT GGAGCGCACC
1051 TCGACGTCGA GCCATGGACC TCTGCACCTT CGTCTTTAGC TACCTGCTGC
1101 CAGTGCTAGT CCTCAGTCTG ACCTATGCGC GTACCCTGCG CTACCTCTGG
1151 CGCACAGTCG ACCCGGTGAC TGCAGGCTCA GGTTCCCAGC GCGCCAAACG
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1201 CAAGGTGACA CGGATGATCA TCATCGTGGC GGTGCTTTTC TGCCTCTGTT

1251 GGATGCCCCA CCACGCGCTT ATCCTCTGCG TGTGGTTTGG TCGCTTCCCG

1301 CTCACGCGTG CCACTTACGC GTTGCGCATC CTTTCACACC TAGTTTCCTA

1351 TGCCAACTCC TGTGTCAACC CCATCGTTTA CGCTCTGGTC TCCAAGCATT

1401 TCCGTAAAGG TTTCCGCAAA ATCTGCGCGG GCCTGCTGCG CCCTGCCCCG

1451 AGGCGAGCTT CGGGCCGAGT GAGCATCCTG GCGCCTGGGA ACCATAGTGG

1501 CAGCATGCTG GAACAGGAAT CCACAGACCT GACACAGGTG AGCGAGGCAG

1551 CCGGGCCCCT TGTCCCACCA CCCGCACTTC CCAACTGCAC AGCCTCGAGT

1601 AGAACCCTGG ATCCGGCTTG TTAAAGGACC AAAGGGCATC TAACAGCTTC

1651 TAGACAGTGT GGCCCGAGGA TCCCTGGGGG TTATGCTTGA ACGTTACAGG

1701 GTTGAGGCTA AAGACTGAGG ATTGATTGTA GGGAACCTCC AGTTATTAAA

1751 CGGTGCGGAT TGCTAGAGGG TGGCATAGTC CTTCAATCCT GGCACCCGAA

1801 AAGCAGATGC AGGAGCAGGA GCAGGAGCAA AGCCAGCCAT GGAGTTTGAG

1851 GCCTGCTTGA ACTACCTGAG ATCCAATAAT AAAACATTTC ATATGCTGTG

1901 AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA

1951 AAAAAAAA

SEQ ID NO: 4: Clone Y107 amino acid sequence (omitting putative intron)

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   1    ATG AAT GGC TCC GGC AGC CAG GGC GCG GAG AAC ACG AGC CAG GAA GGC GGT AGC GGC GGC
   1    Met Asn Gly Ser Gly Ser Gln Gly Ala Glu Asn Thr Ser Gln Glu Gly Gly Ser Gly Gly

  61    TGG CAG CCT GAG GCG GTC CTT GTA CCC CTA TTT TTC GCG CTC ATC TTC CTC GTG GGC ACC
  21    Trp Gln Pro Glu Ala Val Leu Val Pro Leu Phe Phe Ala Leu Ile Phe Leu Val Gly Thr

 121    GTG GGC AAC GCG CTG GTG CTG GCG GTG CTG CTG CGC GGC GGC CAG GCG GTC AGC ACC ACC
  41    Val Gly Asn Ala Leu Val Leu Ala Val Leu Leu Arg Gly Gly Gln Ala Val Ser Thr Thr

 181    AAC CTG TTC ATC CTC AAC CTG GGC GTG GCC GAC CTG TGT TTC ATC CTG TGC TGC GTG CCT
  61    Asn Leu Phe Ile Leu Asn Leu Gly Val Ala Asp Leu Cys Phe Ile Leu Cys Cys Val Pro

 241    TTC CAG GCC ACC ATC TAC ACC CTG GAC GAC TGG GTG TTC GGC TCG CTG CTC TGC AAG GCT
  81    Phe Gln Ala Thr Ile Tyr Thr Leu Asp Asp Trp Val Phe Gly Ser Leu Leu Cys Lys Ala

 301    GTT CAT TTC CTC ATC TTT CTC ACT ATG CAC GCC AGC AGC TTC ACG CTG GCC GCC GTC TCC
 101    Val His Phe Leu Ile Phe Leu Thr Met His Ala Ser Ser Phe Thr Leu Ala Ala Val Ser

 361    CTG GAC AGG TAT CTG GCC ATC CGC TAC CCG CTG CAC TCC CGA GAG TTG CGC ACA CCT CGA
 121    Leu Asp Arg Tyr Leu Ala Ile Arg Tyr Pro Leu His Ser Arg Glu Leu Arg Thr Pro Arg

 421    AAC GCG CTG GCC GCC ATC GGG CTC ATC TGG GGG CTA GCA CTG CTC TTC TCC GGG CCC TAC
 141    Asn Ala Leu Ala Ala Ile Gly Leu Ile Trp Gly Leu Ala Leu Leu Phe Ser Gly Pro Tyr

 481    CTG AGC TAC TAC CGT CAG TCG CAG CTG GCC AAC CTG ACA GTA TGC CAC CCA GCA TGG AGC
 161    Leu Ser Tyr Tyr Arg Gln Ser Gln Leu Ala Asn Leu Thr Val Cys His Pro Ala Trp Ser

 541    GCA CCT CGA CGT CGA GCC ATG GAC CTC TGC ACC TTC GTC TTT AGC TAC CTG CTG CCA GTG
 181    Ala Pro Arg Arg Arg Ala Met Asp Leu Cys Thr Phe Val Phe Ser Tyr Leu Leu Pro Val

 601    CTA GTC CTC AGT CTG ACC TAT GCG CGT ACC CTG CGC TAC CTC TGG CGC ACA GTC GAC CCG
 201    Leu Val Leu Ser Leu Thr Tyr Ala Arg Thr Leu Arg Tyr Leu Trp Arg Thr Val Asp Pro

 661    GTG ACT GCA GGC TCA GGT TCC CAG CGC GCC AAA CGC AAG GTG ACA CGG ATG ATC ATC ATC
 221    Val Thr Ala Gly Ser Gly Ser Gln Arg Ala Lys Arg Lys Val Thr Arg Met Ile Ile Ile

 721    GTG GCG GTG CTT TTC TGC CTC TGT TGG ATG CCC CAC CAC GCG CTT ATC CTC TGC GTG TGG
 241    Val Ala Val Leu Phe Cys Leu Cys Trp Met Pro His His Ala Leu Ile Leu Cys Val Trp

 781    TTT GGT CGC TTC CCG CTC ACG CGT GCC ACT TAC GCG TTG CGC ATC CTT TCA CAC CTA GTT
 261    Phe Gly Arg Phe Pro Leu Thr Arg Ala Thr Tyr Ala Leu Arg Ile Leu Ser His Leu Val

 841    TCC TAT GCC AAC TCC TGT GTC AAC CCC ATC GTT TAC GCT CTG GTC TCC AAG CAT TTC CGT
 281    Ser Tyr Ala Asn Ser Cys Val Asn Pro Ile Val Tyr Ala Leu Val Ser Lys His Phe Arg

 901    AAA GGT TTC CGC AAA ATC TGC GCG GGC CTG CTG CGC CCT GCC CCG AGG CGA GCT TCG GGC
 301    Lys Gly Phe Arg Lys Ile Cys Ala Gly Leu Leu Arg Pro Ala Pro Arg Arg Ala Ser Gly
```

961 CGA GTG AGC ATC CTG GCG CCT GGG AAC CAT AGT GGC AGC ATG CTG GAA CAG GAA TCC ACA
321 Arg Val Ser Ile Leu Ala Pro Gly Asn His Ser Gly Ser Met Leu Glu Gln Glu Ser Thr

1021 GAC CTG ACA CAG GTG AGC GAG GCA GCC GGG CCC CTT GTC CCA CCA CCC GCA CTT CCC AAC
341 Asp Leu Thr Gln Val Ser Glu Ala Ala Gly Pro Leu Val Pro Pro Pro Ala Leu Pro Asn

1081 TGC ACA GCC TCG AGT AGA ACC CTG GAT CCG GCT TGT TAA 1119
361 Cys Thr Ala Ser Ser Arg Thr Leu Asp Pro Ala Cys * 372

SEQ ID NO: 5: **Human GalR2 partial nucleic acid sequence**

```
1    CTGCGCGGCG GCCAGGCGGT CAGCACTACC AACCTGCTCA TCCTTAACCT
51   GGGCGTGGCC GACCTGTGTT TCATCCTGTG CTGCGTGCCC TTCCAGGCCA
101  CCATCTACAC CCTGGACGGC TGGGTGTTCG GCTCGCTGCT GTGCAAGGCG
151  GTGCACTTCC TCATCTTCCT CACCATGCAC GCCAGCAGCT TCACGCTGGC
201  CGCCGTCTCC CTGGACAGGT ATCTGGCCAT CCGCTACCCG CTGCACTCCC
251  GCGAGCTGCG CACGCCTCGA AACGCGCTGG CAGCCATCGG GCTCATCTGG
301  GGGCTGTCGC TGCTCTTCTC CGGGCCCTAC CTGAGCTACT ACCGCCAGTC
351  GCAGCTGGCC AACCTGACCG TGTGCCATCC CGCGTGGAGC GCCCCTCGCC
401  GCCGCGCCAT GGACATCTGC ACCTTCGTCT TCAGCTACCT GCTTCCTGTG
451  CTGGTTCTCG GCCTGACCTA CGCGCGCACC TTGCGCTACC TCTGGCGCGC
501  CGTCGACCCG GTGGCCGCGG GCTCGGGTGC CCGGCGCGCC AAGCGCAAGG
551  TGACACGCAT GATCCTCATC GTGGCCGCGC TCTTCTGCCT CTGCTGGATG
601  CCCCACCACG CGCTCATCCT CTGCGTGTGG TTCGGCCAGT TCCCGCTCAC
651  GCGCGCCACT TATGCGCTTC GCATCCTCTC GCACCTGGTC TCCTACGCCA
701  ACTCCTGCGT CAACCCCATC GTTTACGCGC TGGTCTCCAA GCACTTCCGC
751  AAAGGCTTCC GCACGATCTG CGCGGGCCTG CTGGGCCGTG CCCCAGGCCG
801  AGCCTCGGGC CGTGTGTGCG CTGCCGCGCG GGGCACCCAC AGTGGCAGCG
851  TGTTGGAGCG CGAGTCCAGC GACCTGTTGC ACATGAGCGA GGCGGCGGGG
901  GCCCTTCGTC CCTGCCCCGG CGCTTCCCAG CCATGCATCC TCGAGCCCTG
951  TCCTGGCCCG TCCTGGCAGG GCCCAAAGGC AGGCGACAGC ATCCTGACGG
1001 TTGATGTGGC CTGAAAGCAC TTAGCGGGCG CGCTGGGATG TCACAGAGTT
1051 GGAGTCATTG TTGGGGGACC GTGGGCCGGA ATT
```

SEQ ID NO: 6: Human GalR2 partial amino acid sequence

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1 CTG CGC GGC GGC CAG GCG GTC AGC ACT ACC AAC CTG CTC ATC CTT AAC CTG GGC GTG GCC
(51) Leu Arg Gly Gly Gln Ala Val Ser Thr Thr Asn Leu Leu Ile Leu Asn Leu Gly Val Ala

61 GAC CTG TGT TTC ATC CTG TGC TGC GTG CCC TTC CAG GCC ACC ATC TAC ACC CTG GAC GGC
   Asp Leu Cys Phe Ile Leu Cys Cys Val Pro Phe Gln Ala Thr Ile Tyr Thr Leu Asp Gly

121 TGG GTG TTC GGC TCG CTG CTG TGC AAG GCG GTG CAC TTC CTC ATC TTC CTC ACC ATG CAC
    Trp Val Phe Gly Ser Leu Leu Cys Lys Ala Val His Phe Leu Ile Phe Leu Thr Met His

181 GCC AGC AGC TTC ACG CTG GCC GCC GTC TCC CTG GAC AGG TAT CTG GCC ATC CGC TAC CCG
    Ala Ser Ser Phe Thr Leu Ala Ala Val Ser Leu Asp Arg Tyr Leu Ala Ile Arg Tyr Pro

241 CTG CAC TCC CGC GAG CTG CGC ACG CCT CGA AAC GCG CTG GCA GCC ATC GGG CTC ATC TGG
    Leu His Ser Arg Glu Leu Arg Thr Pro Arg Asn Ala Leu Ala Ala Ile Gly Leu Ile Trp

301 GGG CTG TCG CTG CTC TTC TCC GGG CCC TAC CTG AGC TAC TAC CGC CAG TCG CAG CTG GCC
    Gly Leu Ser Leu Leu Phe Ser Gly Pro Tyr Leu Ser Tyr Tyr Arg Gln Ser Gln Leu Ala

361 AAC CTG ACC GTG TGC CAT CCC GCG TGG AGC GCC CCT CGC CGC CGC GCC ATG GAC ATC TGC
    Asn Leu Thr Val Cys His Pro Ala Trp Ser Ala Pro Arg Arg Arg Ala Met Asp Ile Cys

421 ACC TTC GTC TTC AGC TAC CTG CTT CCT GTG CTG GTT CTC GGC CTG ACC TAC GCG CGC ACC
    Thr Phe Val Phe Ser Tyr Leu Leu Pro Val Leu Val Leu Gly Leu Thr Tyr Ala Arg Thr

481 TTG CGC TAC CTC TGG CGC GCC GTC GAC CCG GTG GCC GCG GGC TCG GGT GCC CGG CGC GCC
    Leu Arg Tyr Leu Trp Arg Ala Val Asp Pro Val Ala Ala Gly Ser Gly Ala Arg Arg Ala

541 AAG CGC AAG GTG ACA CGC ATG ATC CTC ATC GTG GCC GCG CTC TTC TGC CTC TGC TGG ATG
    Lys Arg Lys Val Thr Arg Met Ile Leu Ile Val Ala Ala Leu Phe Cys Leu Cys Trp Met

601 CCC CAC CAC GCG CTC ATC CTC TGC GTG TGG TTC GGC CAG TTC CCG CTC ACG CGC GCC ACT
    Pro His His Ala Leu Ile Leu Cys Val Trp Phe Gly Gln Phe Pro Leu Thr Arg Ala Thr

661 TAT GCG CTT CGC ATC CTC TCG CAC CTG GTC TCC TAC GCC AAC TCC TGC GTC AAC CCC ATC
    Tyr Ala Leu Arg Ile Leu Ser His Leu Val Ser Tyr Ala Asn Ser Cys Val Asn Pro Ile

721 GTT TAC GCG CTG GTC TCC AAG CAC TTC CGC AAA GGC TTC CGC ACG ATC TGC GCG GGC CTG
    Val Tyr Ala Leu Val Ser Lys His Phe Arg Lys Gly Phe Arg Thr Ile Cys Ala Gly Leu

781 CTG GGC CGT GCC CCA GGC CGA GCC TCG GGC CGT GTG TGC GCT GCC GCG CGG GGC ACC CAC
    Leu Gly Arg Ala Pro Gly Arg Ala Ser Gly Arg Val Cys Ala Ala Ala Arg Gly Thr His

841 AGT GGC AGC GTG TTG GAG CGC GAG TCC AGC GAC CTG TTG CAC ATG AGC GAG GCG GCG GGG
    Ser Gly Ser Val Leu Glu Arg Glu Ser Ser Asp Leu Leu His Met Ser Glu Ala Ala Gly

901 GCC CTT CGT CCC TGC CCC GGC GCT TCC CAG CCA TGC ATC CTC GAG CCC TGT CCT GGC CCG
    Ala Leu Arg Pro Cys Pro Gly Ala Ser Gln Pro Cys Ile Leu Glu Pro Cys Pro Gly Pro
```

961 TCC TGG CAG GGC CCA AAG GCA GGC GAC AGC ATC CTG ACG GTT GAT GTG GCC TGA AAGCACT
Ser Trp Gln Gly Pro Lys Ala Gly Asp Ser Ile Leu Thr Val Asp Val Ala *

1022 TAGCGGGCGCGCTGGGATGTCACAGAGTTGGAGTCATTGTTGGGGGACCGTGGGCCGGAATT

What is claimed as new and useful is:

1. A polynucleotide molecule coding for GalR2 comprising SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5, or a variant or fragment thereof.
2. A purified and isolated GalR2 protein comprising SEQ ID NO: 2, SEQ ID NO: 4 and SEQ ID NO: 6 or a variant or fragment thereof.
3. A vector comprising a neucleic acid sequence according to claim 1.
4. A cell transformed or transfected with the vector according to claim 3.
5. A method of identifying a GalR2 receptor agonist or antagonist comprising contacting a membrane prepared from cells according to claim 4 with test material and evaluating affinity of the test material to the GalR2 receptor.

```
   1  TCGACCCACG CGTCCGCTCA AGTCTAAAGC AGAGCGAGTC CCAGGACTTG
  51  AGCGCGGGAA GCGAATGGAG TCAGGGTCAT TCGATTGCAC CTCTCTCGGC
 101  TGCGGGCCGG AGCGGGGTAC CATCCTACAC TCTGGGTGCT CCCTCCTCCT
 151  CCCGTCCCCC GCGCACCCCT GCCCTGGCTC CTGGAGCTCG GCAGTCTCGC
 201  TGGGGCGCTG CAGCGAGGGA GCAGCGTGCT CACCAAGACC CGGACAGCTG
 251  CGGGAGCGGC GTCCACTTTG GTGATACCAT GAATGGCTCC GGCAGCCAGG
 301  GCGCGGAGAA CACGAGCCAG GAAGGCGGTA GCGGCGGCTG GCAGCCTGAG
 351  GCGGTCCTTG TACCCCTATT TTTCGCGCTC ATCTTCCTCG TGGGCACCGT
 401  GGGCAACGCG CTGGTGCTGG CGGTGCTGCT GCGCGGCGGC CAGGCGGTCA
 451  GCACCACCAA CCTGTTCATC CTCAACCTGG GCGTGGCCGA CCTGTGTTTC
 501  ATCCTGTGCT GCGTGCCTTT CCAGGCCACC ATCTACACCC TGGACGACTG
 551  GGTGTTCGGC TCGCTGCTCT GCAAGGCTGT TCATTTCCTC ATCTTTCTCA
 601  CTATGCACGC CAGCAGCTTC ACGCTGGCCG CCGTCTCCCT GGACAGGTAT
 651  CTGGCCATCC GCTACCCGCT GCACTCCCGA GAGTTGCGCA CACCTCGAAA
 701  CGCGCTGGCC GCCATCGGGC TCATCTGGGG GCTAGCACTG CTCTTCTCCG
 751  GGCCCTACCT GAGCTACTAC CGTCAGTCGC AGCTGGCCAA CCTGACAGTA
 801  TGCCACCCAG CATGGAGCGC ACCTCGACGT CGAGCCATGG ACCTCTGCAC
 851  CTTCGTCTTT AGCTACCTGC TGCCAGTGCT AGTCCTCAGT CTGACCTATG
 901  CGCGTACCCT GCGCTACCTC TGGCGCACAG TCGACCCGGT GACTGCAGGC
 951  TCAGGTTCCC AGAGCGCCAA ACGCAAGGTG ACACGGATGA TCATCATCGT
1001  GGCGGTGCTT TTCTGCCTCT GTTGGATGCC CCACCACGCG CTTATCCTCT
1051  GCGTGTGGTT TGGTCGCTTC CCGCTCACGC GTGCCACTTA CGCGTTGCGC
1101  ATCCTTTCAC ACCTAGTTTC CTATGCCAAC TCCTGTGTCA ACCCCATCGT
1151  TTACGCTCTG GTCTCCAAGC ATTTCCGTAA AGGTTTCCGC AAAATCTGCG
1201  CGGGCCTGCT GCGCCCTGCC CCGAGGCGAG CTTCGGGCCG AGTGAGCATC
1251  CTGGCGCCTG GGAACCATAG TGGCAGCATG CTGGAACAGG AATCCACAGA
1301  CCTGACACAG GTGAGCGAGG CAGCCGGGCC CCTTGTCCCA CCACCCGCAC
1351  TTCCCAACTG CACAGCCTCG AGTAGAACCC TGGATCCGGC TTGTTAAAGG
1401  ACCAAAGGGC ATCTAACAGC TTCTAGACAG TGTGGCCCGA GGATCCCTGG
1451  GGGTTATGCT TGAACGTTAC AGGGTTGAGG CTAAAGACTG AGGATTGATT
1501  GTAGGGAACC TCCAGTTATT AAACGGTGCG GATTGCTAGA GGGTGGCATA
1551  GTCCTTCAAT CCTGGCACCC GAAAAGCAGA TGCAGGAGCA GGAGCAGGAG
1601  CAAAGCCAGC CATGGAGTTT GAGGCCTGCT TGAACTACCT GAGATCCAAT
1651  AATAAAACAT TTCATATGCT GTGAAAAAAA AAAAAAAAAA
```

FIGURE 1

```
1     ATG AAT GGC TCC GGC AGC CAG GGC GCG GAG AAC ACG AGC CAG GAA GGC GGT AGC GGC GGC
1     Met Asn Gly Ser Gly Ser Gln Gly Ala Glu Asn Thr Ser Gln Glu Gly Gly Ser Gly Gly

61    TGG CAG CCT GAG GCG GTC CTT GTA CCC CTA TTT TTC GCG CTC ATC TTC CTC GTG GGC ACC
21    Trp Gln Pro Glu Ala Val Leu Val Pro Leu Phe Phe Ala Leu Ile Phe Leu Val Gly Thr

121   GTG GGC AAC GCG CTG GTG CTG GCG GTG CTG CTG CGC GGC GGC CAG GCG GTC AGC ACC ACC
41    Val Gly Asn Ala Leu Val Leu Ala Val Leu Leu Arg Gly Gly Gln Ala Val Ser Thr Thr

181   AAC CTG TTC ATC CTC AAC CTG GGC GTG GCC GAC CTG TGT TTC ATC CTG TGC TGC GTG CCT
61    Asn Leu Phe Ile Leu Asn Leu Gly Val Ala Asp Leu Cys Phe Ile Leu Cys Cys Val Pro

241   TTC CAG GCC ACC ATC TAC ACC CTG GAC GAC TGG GTG TTC GGC TCG CTG CTC TGC AAG GCT
81    Phe Gln Ala Thr Ile Tyr Thr Leu Asp Asp Trp Val Phe Gly Ser Leu Leu Cys Lys Ala

301   GTT CAT TTC CTC ATC TTT CTC ACT ATG CAC GCC AGC AGC TTC ACG CTG GCC GCC GTC TCC
101   Val His Phe Leu Ile Phe Leu Thr Met His Ala Ser Ser Phe Thr Leu Ala Ala Val Ser

361   CTG GAC AGG TAT CTG GCC ATC CGC TAC CCG CTG CAC TCC CGA GAG TTG CGC ACA CCT CGA
121   Leu Asp Arg Tyr Leu Ala Ile Arg Tyr Pro Leu His Ser Arg Glu Leu Arg Thr Pro Arg

421   AAC GCG CTG GCC GCC ATC GGG CTC ATC TGG GGG CTA GCA CTG CTC TTC TCC GGG CCC TAC
141   Asn Ala Leu Ala Ala Ile Gly Leu Ile Trp Gly Leu Ala Leu Leu Phe Ser Gly Pro Tyr

481   CTG AGC TAC TAC CGT CAG TCG CAG CTG GCC AAC CTG ACA GTA TGC CAC CCA GCA TGG AGC
161   Leu Ser Tyr Tyr Arg Gln Ser Gln Leu Ala Asn Leu Thr Val Cys His Pro Ala Trp Ser

541   GCA CCT CGA CGT CGA GCC ATG GAC CTC TGC ACC TTC GTC TTT AGC TAC CTG CTG CCA GTG
181   Ala Pro Arg Arg Arg Ala Met Asp Leu Cys Thr Phe Val Phe Ser Tyr Leu Leu Pro Val

601   CTA GTC CTC AGT CTG ACC TAT GCG CGT ACC CTG CGC TAC CTC TGG CGC ACA GTC GAC CCG
201   Leu Val Leu Ser Leu Thr Tyr Ala Arg Thr Leu Arg Tyr Leu Trp Arg Thr Val Asp Pro

661   GTG ACT GCA GGC TCA GGT TCC CAG AGC GCC AAA CGC AAG GTG ACA CGG ATG ATC ATC ATC
221   Val Thr Ala Gly Ser Gly Ser Gln Ser Ala Lys Arg Lys Val Thr Arg Met Ile Ile Ile

721   GTG GCG GTG CTT TTC TGC CTC TGT TGG ATG CCC CAC CAC GCG CTT ATC CTC TGC GTG TGG
241   Val Ala Val Leu Phe Cys Leu Cys Trp Met Pro His His Ala Leu Ile Leu Cys Val Trp

781   TTT GGT CGC TTC CCG CTC ACG CGT GCC ACT TAC GCG TTG CGC ATC CTT TCA CAC CTA GTT
261   Phe Gly Arg Phe Pro Leu Thr Arg Ala Thr Tyr Ala Leu Arg Ile Leu Ser His Leu Val

841   TCC TAT GCC AAC TCC TGT GTC AAC CCC ATC GTT TAC GCT CTG GTC TCC AAG CAT TTC CGT
281   Ser Tyr Ala Asn Ser Cys Val Asn Pro Ile Val Tyr Ala Leu Val Ser Lys His Phe Arg

901   AAA GGT TTC CGC AAA ATC TGC GCG GGC CTG CTG CGC CCT GCC CCG AGG CGA GCT TCG GGC
301   Lys Gly Phe Arg Lys Ile Cys Ala Gly Leu Leu Arg Pro Ala Pro Arg Arg Ala Ser Gly

961   CGA GTG AGC ATC CTG GCG CCT GGG AAC CAT AGT GGC AGC ATG CTG GAA CAG GAA TCC ACA
321   Arg Val Ser Ile Leu Ala Pro Gly Asn His Ser Gly Ser Met Leu Glu Gln Glu Ser Thr

1021  GAC CTG ACA CAG GTG AGC GAG GCA GCC GGG CCC CTT GTC CCA CCA CCC GCA CTT CCC AAC
341   Asp Leu Thr Gln Val Ser Glu Ala Ala Gly Pro Leu Val Pro Pro Pro Ala Leu Pro Asn

1081  TGC ACA GCC TCG AGT AGA ACC CTG GAT CCG GCT TGT TAA  1119
361   Cys Thr Ala Ser Ser Arg Thr Leu Asp Pro Ala Cys  *   372
```

FIGURE 2

```
   1  CCACTTTGGT GATACCATGA ATGGCTCCGG CAGCCAGGGC GCGGAGAACA
  51  CGAGCCAGGA AGGCGGTAGC GGCGGCTGGC AGCCTGAGGC GGTCCTTGTA
 101  CCCCTATTTT TCGCGCTCAT CTTCCTCGTG GGCACCGTGG GCAACGCGCT
 151  GGTGCTGGCG GTGCTGCTGC GCGGCGGCCA GGCGGTCAGC ACCACCAACC
 201  TGTTCATCCT CAACCTGGGC GTGGCCGACC TGTGTTTCAT CCTGTGCTGC
 251  GTGCCTTTCC AGGCCACCAT CTACACCCTG GACGACTGGG TGTTCGGCTC
 301  GCTGCTCTGC AAGGCTGTTC ATTTCCTCAT CTTTCTCACT ATGCACGCCA
 351  GCAGCTTCAC GCTGGCCGCC GTCTCCCTGG ACAGGTAAAG GACCCAGAAA
 401  GAAACATCCA GTATGCCCGG AGGGATCITG ACTGGAAAAG ACTGAATCCT
 451  GGTCTGGTGA CCTTAGTTCC CTGCCCTTTC ACATCACTTG GACATTCCCA
 501  CAGAAGAGCG GTGAAGAGGC GGTGGTCCTT ATTCTCCTCT GGTTTCCACT
 551  GAGTGCAACA TGTGCGTCCT GAGTACGCTG GAGGGACTCA CAAAATTTCA
 601  GCTTTCTTTA GGAGTTTCCT TGCTGTAGTT TGACCCAAGT CTTCTCCAGG
 651  TTTCTGTCAG AACTCAGGCA TGAGGGATCT GCCTCCCCTG GTTGTCACCA
 701  GAGGATAACA ATCACTGCCC CCAGAAATCC AGACAGATTC TACAACTTTT
 751  AGTCTTCGGT GTTTTGGGGG TGCCCCTTCA CGTGGAGTAG GTCGGTGGCC
 801  ACATTCCCAG GAGTGACAAT AGCCTAGCAG TGAATCCTCT CGCTTAGCTG
 851  ATGCCCCCCC ACTGTCCCCA CAGGTATCTG GCCATCCGCT ACCCGCTGCA
 901  CTCCCGAGAG TTGCGCACAC CTCGAAACGC GCTGGCCGCC ATCGGGCTCA
 951  TCTGGGGGCT AGCACTGCTC TTCTCCGGGC CCTACCTGAG CTACTACCGT
1001  CAGTCGCAGC TGGCCAACCT GACAGTATGC CACCCAGCAT GGAGCGCACC
1051  TCGACGTCGA GCCATGGACC TCTGCACCTT CGTCTTTAGC TACCTGCTGC
1101  CAGTGCTAGT CCTCAGTCTG ACCTATGCGC GTACCCTGCG CTACCTCTGG
1151  CGCACAGTCG ACCCGGTGAC TGCAGGCTCA GGTTCCCAGC GCGCCAAACG
1201  CAAGGTGACA CGGATGATCA TCATCGTGGC GGTGCTTTTC TGCCTCTGTT
1251  GGATGCCCCA CCACGCGCTT ATCCTCTGCG TGTGGTTTGG TCGCTTCCCG
1301  CTCACGCGTG CCACTTACGC GTTGCGCATC CTTTCACACC TAGTTTCCTA
1351  TGCCAACTCC TGTGTCAACC CCATCGTTTA CGCTCTGGTC TCCAAGCATT
1401  TCCGTAAAGG TTTCCGCAAA ATCTGCGCGG GCCTGCTGCG CCCTGCCCCG
1451  AGGCGAGCTT CGGGCCGAGT GAGCATCCTG GCGCCTGGGA ACCATAGTGG
1501  CAGCATGCTG GAACAGGAAT CCACAGACCT GACACAGGTG AGCGAGGCAG
1551  CCGGGCCCCT TGTCCCACCA CCCGCACTTC CCAACTGCAC AGCCTCGAGT
1601  AGAACCCTGG ATCCGGCTTG TTAAAGGACC AAAGGGCATC TAACAGCTTC
```

FIGURE 3

```
1651  TAGACAGTGT GGCCCGAGGA TCCCTGGGGG TTATGCTTGA ACGTTACAGG
1701  GTTGAGGCTA AAGACTGAGG ATTGATTGTA GGGAACCTCC AGTTATTAAA
1751  CGGTGCGGAT TGCTAGAGGG TGGCATAGTC CTTCAATCCT GGCACCCGAA
1801  AAGCAGATGC AGGAGCAGGA GCAGGAGCAA AGCCAGCCAT GGAGTTTGAG
1851  GCCTGCTTGA ACTACCTGAG ATCCAATAAT AAAACATTTC ATATGCTGTG
1901  AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
1951  AAAAAAAA
```

FIGURE 4

```
1     ATG AAT GGC TCC GGC AGC CAG GGC GCG GAG AAC ACG AGC CAG GAA GGC GGT AGC GGC GGC
1     Met Asn Gly Ser Gly Ser Gln Gly Ala Glu Asn Thr Ser Gln Glu Gly Gly Ser Gly Gly

61    TGG CAG CCT GAG GCG GTC CTT GTA CCC CTA TTT TTC GCG CTC ATC TTC CTC GTG GGC ACC
21    Trp Gln Pro Glu Ala Val Leu Val Pro Leu Phe Phe Ala Leu Ile Phe Leu Val Gly Thr

121   GTG GGC AAC GCG CTG GTG CTG GCG GTG CTG CTG CGC GGC GGC CAG GCG GTC AGC ACC ACC
41    Val Gly Asn Ala Leu Val Leu Ala Val Leu Leu Arg Gly Gly Gln Ala Val Ser Thr Thr

181   AAC CTG TTC ATC CTC AAC CTG GGC GTG GCC GAC CTG TGT TTC ATC CTG TGC TGC GTG CCT
61    Asn Leu Phe Ile Leu Asn Leu Gly Val Ala Asp Leu Cys Phe Ile Leu Cys Cys Val Pro

241   TTC CAG GCC ACC ATC TAC ACC CTG GAC GAC TGG GTG TTC GGC TCG CTG CTC TGC AAG GCT
81    Phe Gln Ala Thr Ile Tyr Thr Leu Asp Asp Trp Val Phe Gly Ser Leu Leu Cys Lys Ala

301   GTT CAT TTC CTC ATC TTT CTC ACT ATG CAC GCC AGC AGC TTC ACG CTG GCC GCC GTC TCC
101   Val His Phe Leu Ile Phe Leu Thr Met His Ala Ser Ser Phe Thr Leu Ala Ala Val Ser

361   CTG GAC AGG TAT CTG GCC ATC CGC TAC CCG CTG CAC TCC CGA GAG TTG CGC ACA CCT CGA
121   Leu Asp Arg Tyr Leu Ala Ile Arg Tyr Pro Leu His Ser Arg Glu Leu Arg Thr Pro Arg

421   AAC GCG CTG GCC GCC ATC GGG CTC ATC TGG GGG CTA GCA CTG CTC TTC TCC GGG CCC TAC
141   Asn Ala Leu Ala Ala Ile Gly Leu Ile Trp Gly Leu Ala Leu Leu Phe Ser Gly Pro Tyr

481   CTG AGC TAC TAC CGT CAG TCG CAG CTG GCC AAC CTG ACA GTA TGC CAC CCA GCA TGG AGC
161   Leu Ser Tyr Tyr Arg Gln Ser Gln Leu Ala Asn Leu Thr Val Cys His Pro Ala Trp Ser

541   GCA CCT CGA CGT CGA GCC ATG GAC CTC TGC ACC TTC GTC TTT AGC TAC CTG CTG CCA GTG
181   Ala Pro Arg Arg Arg Ala Met Asp Leu Cys Thr Phe Val Phe Ser Tyr Leu Leu Pro Val

601   CTA GTC CTC AGT CTG ACC TAT GCG CGT ACC CTG CGC TAC CTC TGG CGC ACA GTC GAC CCG
201   Leu Val Leu Ser Leu Thr Tyr Ala Arg Thr Leu Arg Tyr Leu Trp Arg Thr Val Asp Pro

661   GTG ACT GCA GGC TCA GGT TCC CAG CGC GCC AAA CGC AAG GTG ACA CGG ATG ATC ATC ATC
221   Val Thr Ala Gly Ser Gly Ser Gln Arg Ala Lys Arg Lys Val Thr Arg Met Ile Ile Ile

721   GTG GCG GTG CTT TTC TGC CTC TGT TGG ATG CCC CAC CAC GCG CTT ATC CTC TGC GTG TGG
241   Val Ala Val Leu Phe Cys Leu Cys Trp Met Pro His His Ala Leu Ile Leu Cys Val Trp

781   TTT GGT CGC TTC CCG CTC ACG CGT GCC ACT TAC GCG TTG CGC ATC CTT TCA CAC CTA GTT
261   Phe Gly Arg Phe Pro Leu Thr Arg Ala Thr Tyr Ala Leu Arg Ile Leu Ser His Leu Val

841   TCC TAT GCC AAC TCC TGT GTC AAC CCC ATC GTT TAC GCT CTG GTC TCC AAG CAT TTC CGT
281   Ser Tyr Ala Asn Ser Cys Val Asn Pro Ile Val Tyr Ala Leu Val Ser Lys His Phe Arg

901   AAA GGT TTC CGC AAA ATC TGC GCG GGC CTG CTG CGC CCT GCC CCG AGG CGA GCT TCG GGC
301   Lys Gly Phe Arg Lys Ile Cys Ala Gly Leu Leu Arg Pro Ala Pro Arg Arg Ala Ser Gly

961   CGA GTG AGC ATC CTG GCG CCT GGG AAC CAT AGT GGC AGC ATG CTG GAA CAG GAA TCC ACA
321   Arg Val Ser Ile Leu Ala Pro Gly Asn His Ser Gly Ser Met Leu Glu Gln Glu Ser Thr

1021  GAC CTG ACA CAG GTG AGC GAG GCA GCC GGG CCC CTT GTC CCA CCA CCC GCA CTT CCC AAC
341   Asp Leu Thr Gln Val Ser Glu Ala Ala Gly Pro Leu Val Pro Pro Pro Ala Leu Pro Asn

1081  TGC ACA GCC TCG AGT AGA ACC CTG GAT CCG GCT TGT TAA  1119
361   Cys Thr Ala Ser Ser Arg Thr Leu Asp Pro Ala Cys  *   372
```

FIGURE 5

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   1  CTGCGCGGCG GCCAGGCGGT CAGCACTACC AACCTGCTCA TCCTTAACCT
  51  GGGCGTGGCC GACCTGTGTT TCATCCTGTG CTGCGTGCCC TTCCAGGCCA
 101  CCATCTACAC CCTGGACGGC TGGGTGTTCG GCTCGCTGCT GTGCAAGGCG
 151  GTGCACTTCC TCATCTTCCT CACCATGCAC GCCAGCAGCT TCACGCTGGC
 201  CGCCGTCTCC CTGGACAGGT ATCTGGCCAT CCGCTACCCG CTGCACTCCC
 251  GCGAGCTGCG CACGCCTCGA AACGCGCTGG CAGCCATCGG GCTCATCTGG
 301  GGGCTGTCGC TGCTCTTCTC CGGGCCCTAC CTGAGCTACT ACCGCCAGTC
 351  GCAGCTGGCC AACCTGACCG TGTGCCATCC CGCGTGGAGC GCCCCTCGCC
 401  GCCGCGCCAT GGACATCTGC ACCTTCGTCT TCAGCTACCT GCTTCCTGTG
 451  CTGGTTCTCG GCCTGACCTA CGCGCGCACC TTGCGCTACC TCTGGCGCGC
 501  CGTCGACCCG GTGGCCGCGG GCTCGGGTGC CCGGCGCGCC AAGCGCAAGG
 551  TGACACGCAT GATCCTCATC GTGGCCGCGC TCTTCTGCCT CTGCTGGATG
 601  CCCCACCACG CGCTCATCCT CTGCGTGTGG TTCGGCCAGT TCCCGCTCAC
 651  GCGCGCCACT TATGCGCTTC GCATCCTCTC GCACCTGGTC TCCTACGCCA
 701  ACTCCTGCGT CAACCCCATC GTTTACGCGC TGGTCTCCAA GCACTTCCGC
 751  AAAGGCTTCC GCACGATCTG CGCGGGCCTG CTGGGCCGTG CCCCAGGCCG
 801  AGCCTCGGGC CGTGTGTGCG CTGCCGCGCG GGGCACCCAC AGTGGCAGCG
 851  TGTTGGAGCG CGAGTCCAGC GACCTGTTGC ACATGAGCGA GGCGGCGGGG
 901  GCCCTTCGTC CCTGCCCCGG CGCTTCCCAG CCATGCATCC TCGAGCCCTG
 951  TCCTGGCCCG TCCTGGCAGG GCCCAAAGGC AGGCGACAGC ATCCTGACGG
1001  TTGATGTGGC CTGAAAGCAC TTAGCGGGCG CGCTGGGATG TCACAGAGTT
1051  GGAGTCATTG TTGGGGGACC GTGGGCCGGA ATT
```

FIGURE 6

```
  1 CTG CGC GGC GGC CAG GCG GTC AGC ACT ACC AAC CTG CTC ATC CTT AAC CTG GGC GTG GCC
(51) Leu Arg Gly Gly Gln Ala Val Ser Thr Thr Asn Leu Leu Ile Leu Asn Leu Gly Val Ala

 61 GAC CTG TGT TTC ATC CTG TGC TGC GTG CCC TTC CAG GCC ACC ATC TAC ACC CTG GAC GGC
    Asp Leu Cys Phe Ile Leu Cys Cys Val Pro Phe Gln Ala Thr Ile Tyr Thr Leu Asp Gly

121 TGG GTG TTC GGC TCG CTG CTG TGC AAG GCG GTG CAC TTC CTC ATC TTC CTC ACC ATG CAC
    Trp Val Phe Gly Ser Leu Leu Cys Lys Ala Val His Phe Leu Ile Phe Leu Thr Met His

181 GCC AGC AGC TTC ACG CTG GCC GCC GTC TCC CTG GAC AGG TAT CTG GCC ATC CGC TAC CCG
    Ala Ser Ser Phe Thr Leu Ala Ala Val Ser Leu Asp Arg Tyr Leu Ala Ile Arg Tyr Pro

241 CTG CAC TCC CGC GAG CTG CGC ACG CCT CGA AAC GCG CTG GCA GCC ATC GGG CTC ATC TGG
    Leu His Ser Arg Glu Leu Arg Thr Pro Arg Asn Ala Leu Ala Ala Ile Gly Leu Ile Trp

301 GGG CTG TCG CTG CTC TTC TCC GGG CCC TAC CTG AGC TAC TAC CGC CAG TCG CAG CTG GCC
    Gly Leu Ser Leu Leu Phe Ser Gly Pro Tyr Leu Ser Tyr Tyr Arg Gln Ser Gln Leu Ala

361 AAC CTG ACC GTG TGC CAT CCC GCG TGG AGC GCC CCT CGC CGC CGC GCC ATG GAC ATC TGC
    Asn Leu Thr Val Cys His Pro Ala Trp Ser Ala Pro Arg Arg Arg Ala Met Asp Ile Cys

421 ACC TTC GTC TTC AGC TAC CTG CTT CCT GTG CTG GTT CTC GGC CTG ACC TAC GCG CGC ACC
    Thr Phe Val Phe Ser Tyr Leu Leu Pro Val Leu Val Leu Gly Leu Thr Tyr Ala Arg Thr

481 TTG CGC TAC CTC TGG CGC GCC GTC GAC CCG GTG GCC GCG GGC TCG GGT GCC CGG CGC GCC
    Leu Arg Tyr Leu Trp Arg Ala Val Asp Pro Val Ala Ala Gly Ser Gly Ala Arg Arg Ala

541 AAG CGC AAG GTG ACA CGC ATG ATC CTC ATC GTG GCC GCG CTC TTC TGC CTC TGC TGG ATG
    Lys Arg Lys Val Thr Arg Met Ile Leu Ile Val Ala Ala Leu Phe Cys Leu Cys Trp Met

601 CCC CAC CAC GCG CTC ATC CTC TGC GTG TGG TTC GGC CAG TTC CCG CTC ACG CGC GCC ACT
    Pro His His Ala Leu Ile Leu Cys Val Trp Phe Gly Gln Phe Pro Leu Thr Arg Ala Thr

661 TAT GCG CTT CGC ATC CTC TCG CAC CTG GTC TCC TAC GCC AAC TCC TGC GTC AAC CCC ATC
    Tyr Ala Leu Arg Ile Leu Ser His Leu Val Ser Tyr Ala Asn Ser Cys Val Asn Pro Ile

721 GTT TAC GCG CTG GTC TCC AAG CAC TTC CGC AAA GGC TTC CGC ACG ATC TGC GCG GGC CTG
    Val Tyr Ala Leu Val Ser Lys His Phe Arg Lys Gly Phe Arg Thr Ile Cys Ala Gly Leu

781 CTG GGC CGT GCC CCA GGC CGA GCC TCG GGC CGT GTG TGC GCT GCC GCG CGG GGC ACC CAC
    Leu Gly Arg Ala Pro Gly Arg Ala Ser Gly Arg Val Cys Ala Ala Ala Arg Gly Thr His

841 AGT GGC AGC GTG TTG GAG CGC GAG TCC AGC GAC CTG TTG CAC ATG AGC GAG GCG GCG GGG
    Ser Gly Ser Val Leu Glu Arg Glu Ser Ser Asp Leu Leu His Met Ser Glu Ala Ala Gly

901 GCC CTT CGT CCC TGC CCC GGC GCT TCC CAG CCA TGC ATC CTC GAG CCC TGT CCT GGC CCG
    Ala Leu Arg Pro Cys Pro Gly Ala Ser Gln Pro Cys Ile Leu Glu Pro Cys Pro Gly Pro

961 TCC TGG CAG GGC CCA AAG GCA GGC GAC AGC ATC CTG ACG GTT GAT GTG GCC TGA AAGCACT
    Ser Trp Gln Gly Pro Lys Ala Gly Asp Ser Ile Leu Thr Val Asp Val Ala  *

1022 TAGCGGGCGCGCTGGGATGTCACAGAGTTGGAGTCATTGTTGGGGGACCGTGGGCCGGAATT
```

FIGURE 7

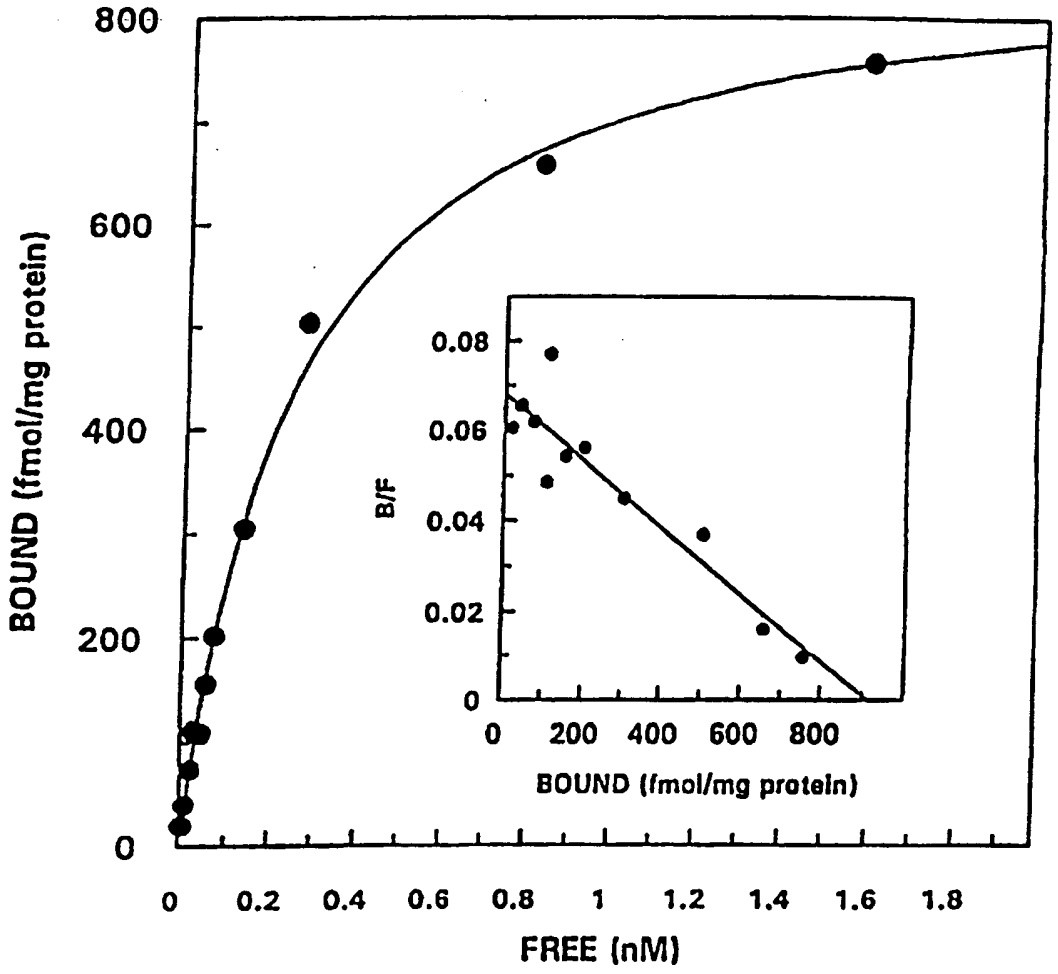
800
600
400
200
0
BOUND (fmol/mg protein)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
FREE (nM)
0.08
0.06
0.04
0.02
0
B/F
0
200
400
600
800
BOUND (fmol/mg protein)

FIGURE 8

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(57) Abstract

The present invention provides a novel galanin receptor protein, the GalR2 receptor. Also provided are the nucleic acid sequences encoding this novel receptor protein as well as methods for using this protein and its nucleic acid sequence, and methods useful for developing and identifying compounds for the treatment of diseases and disorders in which galanin is implicated. The importance of this discovery is manifested in the effects of galanin, which include antinociceptive activity, smooth muscle contraction, cardiovascular activity, pituitary hormone release, cognition, and increased food intake. Thus, this receptor protein is useful for screening for galanin agonist and antagonist activity for controlling these conditions.

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B. FIELDS SEARCHED

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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A	EP 0 711 830 A (TAKEDA CHEMICAL INDUSTRIES LTD) 15 May 1996 cited in the application see the whole document ---	1-5
A	WO 95 22608 A (RHONE POULENC RORER SA ;AMIRANOFF BRIGITTE (FR); HABERT ORTOLI EST) 24 August 1995 cited in the application see the whole document --- -/--	1-5

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1

Date of the actual completion of the international search	Date of mailing of the international search report
12 January 1998	22/01/1998

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A	WYNICK D ET AL: "Characterization of a high-affinity galanin receptor in the rat anterior pituitary: absence of biological effect and reduced membrane binding of the antagonist M15 differentiate it from the brain/gut receptor." PROC NATL ACAD SCI U S A, MAY 1 1993, 90 (9) P4231-5, UNITED STATES, XP002051694 cited in the application	1-5

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Int tional Application No
PCT/US 97/09787

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